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NOTICE

Regarding the Courses of Instruction proposed to be given by the Liverpool School of Tropical Medicine, and the Examinations for the Diploma of Tropical Medicine arranged to be held by the University of Liverpool during 1908 (subject to such alteration as may hereinafter be decided upon),

Lent Term begins January 14.
Lent Examination, March 23.
Summer Term begins May 1.
Summer Examination, July 13.
Autumn Term begins October 1.
Autumn Examination, December 14.

The full Course of Instruction is open to all qualified medical men, and the examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Ten guineas.

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For prospectus and further information, application should be made to the Dean of the Medical Faculty, University of Liverpool.

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Date of Diploma	
1906	Adie, Joseph Rosamond
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1905	Anderson, Catherine Elmslie
1906	Arnold, Frank Arthur
1904	Augustine, Henry Joshua
1906	Bate, John Brabant
1904	Bennett, Arthur King
1906	Bennetts, Harold Graves
1907	Bond, Ashton
1907	Branch, Stanley
1905	Brown, Alexander
1904	Bruce, William James
1904	Byrne, John Scott
1905	Caldwell, Thomas Cathcart
1906	Carter, Robert Markham
1906	Chisholm, James Alexander
1904	Clayton, Thomas Morrison
1906	Clements, Robert William
1907	Collinson, Walter Julius
1905	Critien, Attilio
1904	Dalziel, John McEwen
1907	Davey, John Bernard
1904	Dee, Peter
1907	Donaldson, Anson Scott
1906	Dundas, James
1906	Faichnie, Norman
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1907	Hiscock, Robert Carroll
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz
1906	Jeffreys, Herbert Castelman

Date of Diploma	
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1907	Le Fanu, George Ernest Hugh
1905	Macfarlane, Robert Maxwell
1906	Mackenzie, Donald Francis
1907	Mackey, Charles
1904	Maclurkin, Alfred Robert
1905	Maddock, Edward Cecil Gordon
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1904	McConnell, Robert Ernest
1905	Moore, James Jackson
1904	Nicholson, James Edward
1905	Nightingale, Samuel Shore
1906	Pailthorpe, Mary Elizabeth
1906	Palmer, Harold Thornbury
1906	Pearse, Albert
1904	Philipson, Nicholas
1905	Radcliffe, Percy Alexander Hurst
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1906	Sampey, Alexander William
1904	Sharman, Eric Harding
1906	Smithson, Arthur Ernest
1906	Taylor, Joseph van Someron
1906	Taylor, William Irwin
1904	Thomson, Frank Wyville
1906	Tynan, Edward Joseph
1907	Vallance, Hugh
1904	Walker, George Francis Clegg
1906	Watson, Cecil Francis
1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1905	Young, John Cameron

EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which have been issued since 1899, will be followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the first number of the second volume.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. Altogether twenty-one Memoirs, besides other works, have been published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905; and there is no reason to suppose that this rate of production by the workers of the School alone will diminish in the future. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine or Hygiene may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

ATOXYL AND TRYPANOSOMIASIS

BY

SIR RUBERT BOYCE, F.R.S.,

AND

ANTON BREINL, M.U.D. PRAG.

(Received for publication January 20th, 1908)

Early observations upon the use of Arsenic in the treatment of Fly disease

The brilliant discovery by the late Dr. Dutton in 1901 of the presence of trypanosomes in the blood of a patient under the care of Dr. Forde of Bathurst, to which he gave the name of *Trypanosoma gambiense*, the finding very shortly afterwards, by an expedition sent out to Africa under the auspices of the Royal Society and Colonial Office, composed of CASTELLANI, BRUCE, NABARRO and LOW, that sleeping sickness was caused by the same parasite (*Trypanosoma gambiense*), stimulated investigation throughout the civilised world into the life history of this group of haematozoa, their mode of action in the blood and tissues of man and animals, and the effect of various drugs upon them.

During the year 1907 very material progress has been made in the treatment of sleeping sickness, and it appears to us that the time is a suitable one in which to review the history of how arsenic and its compounds came to be employed, and to state the results of the treatment with this and allied drugs, in the light of the great experience gained in 1907.

There is no doubt that for a very long time Arsenic has been looked upon as a remedy useful in trypanosomiasis in animals. Long before the nature of sleeping sickness was understood there existed much speculation with regard to the nature and treatment of *Tsetse Fly disease* in horses and cattle. First and foremost among those who suggested *Arsenic* as a means of treating this disease in animals stands the great observer and explorer Dr. Livingstone.

DAVID LIVINGSTONE, in a letter (dated March 22, 1858) to the *British Medical Journal* of May 1st, 1858, mentioned that it had occurred to him in about the year 1847-8 to use Arsenic in the disease which followed the bite of the tsetse fly. He mentions how he tried the drug on a mare.

In thanking Mr. BRAID for some remarks (published in the *British Medical Journal*) Livingstone states 'that though my hopes are not sanguine, I still mean to try the remedy, if opportunity offers. Our instructions require us to examine the whole subject carefully, and the results will be communicated to the Royal Society.'

In the *British Medical Journal* of February 13, 1858, there appears a letter from Mr. Braid, dated from Manchester, February 6, 1858, in which he says: 'On reading the interesting facts communicated by Livingstone, one of the most notable is his narrative of the remarkable and fatal phenomena manifested in oxen and sheep from the bite of the tsetse fly. It immediately occurred to me that it would be highly interesting to institute some experiments with the view of discovering a remedy for this curious and fatal malady, and my mind immediately reverted to the prophylactic powers of arsenic against the poison of the most venomous reptiles . . . .'

Braid then quotes Dr. Honigberger's case of a fakir who was an Arsenic eater, and who ascribed to this reason his immunity to the bite of the serpent.

Such at any rate was the source from which the idea developed of *giving small doses of Arsenic to oxen bitten by the tsetse fly.*

Surgeon-Major RANKING had been struck with the similarity existing between the disease Surra in horses and mules and malaria, and recommended a treatment similar to that used in malaria, but although he freely used *Quinine*, we find no evidence that he employed Arsenic.

Use of Arsenious acid and Sodium arseniate

We then come to the period of definite organised experiments, and foremost amongst investigators stands Bruce, who added an immense number of new facts to our very scanty knowledge of trypanosomiasis. BRUCE, in his classical report entitled 'Further Report on the Tsetse Fly disease or Nagana in Zululand' (May 1896), gives a very useful and detailed account of the effect of *Arsenic* as a curative and prophylactic agent.

The experiments he set on foot demonstrated that *Arsenic* had a material effect, and that it was capable of driving haematozoa out of the blood; as Col. Bruce himself states, the drug '*undoubtedly markedly modified the course of the disease.*' As a prophylactic agent, he argued that if Arsenic modified the course of the disease,

it seemed probable therefore that the disease would be prevented altogether if the Arsenic were given for some time previous to the animals being exposed to the infection.

From experiments set on foot to determine whether Arsenic had a prophylactic action, he concludes that it would be quite useless as a prophylactic agent, but that it was useful in prolonging life, and especially in the 'Fly Country,' after the disease had begun. Following upon Bruce's observations come those of LINGARD (1893).

LINGARD (Report on Horse Surra, vol. I, p. 104, 1893) mentions in connection with his experimental enquiry into the treatment of Surra, that Pottinger had tried Arsenic without any appreciable effect. Lingard then conducted himself a series of experiments upon numerous animals with *Arsenic compounds*, *Cinchona alkaloids*, *Arsenic* alone, and also *Mercury perchloride*. The results showed in the case of *Mercury perchloride* that there was no diminution in the number or activity of the parasites in the blood, and that they continued to be present in the blood for three days following the injection of the drug.

Combined treatment with Arsenic and the Cinchona alkaloids

With regard to the treatment with *Cinchona alkaloids and Arsenic*, we quote Lingard's own words (l. c.):—'In the case of two animals, it was decided to attempt curative treatment, although it was recognised that the animal had already been suffering from the disease for a period of at least twenty-three days. It was thought advisable to commence the treatment when the number of haematozoa in the blood should be on the decline, and accordingly on the 14th June, the haematozoa being few, treatment with *Cinchona alkaloids and Arsenic* in large doses was commenced. On the 20th the haematozoa disappeared from the blood, but the temperature and respirations remained above normal. The general symptoms of Surra increased up to the 24th; oedema and swelling of the limbs, sheath and under the abdomen increased in extent. The animal steadily losing condition, the muscles wasting, and the gait staggering and uncertain. On the 25th the animal had decidedly improved, and it was noticed that the oedema of the sheath and abdomen was distinctly less in amount; the animal seemed brighter and moved freely about the loose box.

'This amelioration of the symptoms was gradually progressive, and on the 2nd July the oedema under the belly had completely

disappeared. On the 3rd July, however, the temperature began to rise steadily, and it continued high until the 9th, notwithstanding the fact that no haematozoa were present in the blood. During this time, the animal presented symptoms, which might be accounted for by the continued high temperature, although on the 6th symptoms of kidney disorder presented themselves, followed by epithelial casts and a small number of blood corpuscles in the urine. The large doses of Arsenic and Alkaloids have been persisted in, notwithstanding the intestinal and hepatic derangements, as it was feared that the high temperature indicated the probable return of the Surra haematozoon. On the 9th there were symptoms of gastro-intestinal catarrh, and taking these complications into consideration, as well as the fact that the haematozoa had been absent from the blood for a period of twenty days, it was decided to discontinue the Arsenic and Alkaloids entirely, and a laxative and simple febrifuge was substituted.

'From the 10th to the 14th all went well, and the animal seemed to improve, the temperature remained low, and the animal became much brighter and fed well. On the 15th, however, he was dull, and the respiration and pulse had increased in frequency, the visible mucous membranes becoming of a dirty-yellow colour and petechiated. On the 16th, although his appearance was much brighter, the respiration had risen very high, and the heart's action was irritable; the symptoms of gastro-intestinal catarrh and the kidney disorder had entirely disappeared. On the 17th the animal appeared much in the same condition, but the respirations were less although the temperature was a little higher. On this date one Surra haematozoon was discovered in two cover-glass specimens of blood, and this was the first organism observed during a period of twenty-eight days. The previous treatment of Arsenic and Cinchona alkaloids was at once resumed. On the 18th the animal had not altered much in appearance, the respirations were greatly improved in character and the pulse in frequency, but the temperature had risen. The blood contained a few haematozoa, but not so active in their movements as the one noticed the day before. On the 19th the condition was worse, the haematozoa very numerous in the blood, and the temperature much elevated, viz., 41° C. On the 20th the appetite was normal, but otherwise the animal did not manifest any improvement; only two haematozoa were observed in two cover-glass

specimens of the blood, and these were exceedingly torpid. On the 21st the haematozoa were entirely absent, and from this date the animal appeared unable to throw off the effect which the return of the haematozoon had given rise to, and he gradually became weaker, and died on the 29th of July, although the haematozoa were absent during the last nine days.

'It was decided in this case to try again the effect of the mixed Cinchona alkaloids and Arsenic on the haematozoon, as it had shown promise of efficacy in that of Horse No. LXV, in which case after a short exhibition of the drugs, the haematozoon was kept in subjection for a period of twenty-seven days. Consequently as a fresh trial was resolved upon, the animal was first put upon small doses, beginning with one drachm of the Alkaloids and four grains of Arsenic, given in the form of the *Liq. arsenicalis.* On the morning following the first dose, the temperature was found to have fallen over a degree, and the haematozoon was absent from the blood, the temperature remained low, and the organism absent for the next five days, during which period two doses of the medicine were administered daily.

'On reference to the Chart, it will be seen that on the ninth day of the disease the haematozoon again appeared in the blood, consequently on the tenth the doses of Alkaloids and Arsenic were increased to one and a half drachms and five grains respectively; these were continued for a period of eight days, during which time the haematozoon varied in numbers between few and very numerous. On the eighteenth day of the disease a further increase in the doses was agreed upon, and during the next four days they were put up to Alkaloids two and a half drachms and Arsenic grains vi, and for the latter half of this period the organism was absent from the blood, but re-appeared on the twenty-second day, and remained on the twenty-third at few. On the former date the Alkaloids were increased to three drachms, and the Arsenic to grains viii, and from the twenty-third to the thirty-fifth day of the disease, on which latter date the animal succumbed, the haematozoon was entirely absent from the blood. During the thirty-two days the animal was under treatment, three hundred and fifty-five grains of Arsenic in the form of *Liquor arsenicalis*, and one hundred and fifty-four and a half drachms of the mixed *Cinchona alkaloids* were administered.'

He further quotes the case of an animal belonging to the Bombay

Tramway Company. 'On the morning of the 7th October, 1892, the "Surra" haematozoon was discovered on microscopical examination of its blood. On admission to the Laboratory Hospital the following note was made:—"Bay gelding, aged five years; appears to be in perfect health, and in splendid condition." On this date the haematozoon was absent from the blood, while for the next two days it was swarming: specific treatment was deferred, a simple febrifuge draught being given to try and reduce the temperature. On the evening of the 12th October, the organism was only present in small numbers in the blood, and the temperature had decreased considerably, as had also the pulse. It was decided to again attempt the destruction of the haematozoon by the exhibition of Cinchona Alkaloids and Arsenic, commencing in this instance with large doses (viz., Alkaloid drs. iii, and Arsenic grains iv, *bis die*). The reason why large doses of the former were exhibited was that few haematozoa remained in the blood, and in order to decide whether their destruction could be compassed by the drug, the dose was so regulated as to ensure its having a fair trial. No marked change took place in the animal's condition, nor in the number of the organisms present, until the 16th instant, when the haematozoa became numerous, the temperature rose considerably, respirations were increased in number, and it was noticed that the sheath was swollen; the treatment was continued, the dose of Arsenic having been gradually raised to six grains. On the 17th the organisms had again fallen to few, but the temperature and pulse were still above normal. On this date it was decided to try the effect of full doses of *Quinine*, as this drug has been said to be of much value when exhibited in this manner. Ranking has stated that "by the employment of full doses of *Quinine and Arsenic* he has been able to cure the disease." It appeared from our previous experiments on the use of similar drugs, that it would be futile to attempt the destruction of the haematozoa by giving only the full medicinal dose, and we considered it to be advisable therefore to commence with a dose of drs. iv of quinine, and arsenic grains xii, in the 24 hours, carefully watching its effects, and in case of any untoward symptom appearing, to discontinue its use.'

Sodium arseniate

LAVERAN and MESNIL in 1902 published in the Annales de l'Institut Pasteur their researches 'Sur le Traitement et la prevention

du Nagana.' They obtained their best results by using *sodium arseniate* subcutaneously; with this drug they were able to bring about a quick disappearance of the parasites from the blood. The lives of the animals were prolonged, but a permanent cure was not effected. These observers also used *Sodium cacodylate, Sublimate of Mercury*, various *salts of Silver*, but without effect.

Serum

They were also led to try the effects of injecting *human serum* into ngana-infected rats and mice; they were led to do so as man was not susceptible to ngana. A marked improvement followed the injection of human serum; the duration of the disease was prolonged, but a real cure could not be effected.

E. J. MOORE, in a paper upon the beneficial effects of *Sodium arseniate* employed hypodermically in tsetse-fly disease, and published in the *Lancet*, Vol. II, 1904, records the most marked beneficial effects with large doses, and states that he would also recommend it for humans.

CHICHESTER, who collaborated with him in a letter which he sent to Sir Patrick Manson dated May 5th, 1904, and published in the *Lancet* under the title of 'Arsenic in the treatment of Trypanosomiasis in Cattle in Nigeria,' says that 'he used Arsenic hypodermically and produced most wonderful effects.' He adds that the experiment is not over, but says that 'I do not think it wise to wait longer. I tell you what I have found, and you may perhaps think it wise to ask others to try the same treatment, especially in those parts where it seems a scourge to human beings.'

THOMAS, in 1903, whilst at the MacGill University, in Canada, repeated some of the experiments upon the action of Arsenic on ngana-infected animals, and on joining the Liverpool School of Tropical Medicine, in August of the same year, immediately started extensive investigations upon the action of drugs upon trypanosomiasis. Amongst them he used *Sodium arseniate*, and with this drug his results were similar to those of Laveran. 'Many of the rats either died from the disease, were killed by the drug, or succumbed to an extensive ulceration around the site of injection.' Thomas's own words, taken from his paper entitled 'Some experiments in the treatment of trypanosomiasis' (*British Medical Journal*, May 27, 1905) were: 'Arsenic in the treatment of trypanosomiasis in animals seems rather to mitigate the disease, to cause the parasite to

apparently disappear from the animal's blood, and to prolong the life of the animal. Unfortunately the usual history is that if treatment be discontinued, even after a prolonged course of the drug, the animal, after a varying length of time, once more exhibits the symptoms, and finally succumbs to the disease. Undoubtedly some of the more resistant animals do recover.' Thomas then quotes the view of Laveran and Mesnil (Trypanosomes et Trypanosomiases) that Arsenic kills the parasites which are free in the blood, but that when once the drug is eliminated or fixed in the tissues, the surviving parasites commence to multiply and the organisms once more reappear in the peripheral circulation unless another injection of Arsenic be given. On the administration of a second dose the parasites disappear only to reappear, and even though treatment be kept up, the majority of the animals succumb either from the disease or from the toxic effects of the drug. Some of the animals, such as rats and dogs, have been cured. Laveran also records the extensive necrosis and the pain which is apt to follow the administration of Arsenious acid.

THOMAS and BREINL, in a paper upon 'Trypanosomiasis and Sleeping Sickness,' published as Memoir XVI, 1905, of the Liverpool School of Tropical Medicine, report fully upon the treatment. *Sodium arseniate* was found the most useful form of Arsenic, but the usual disadvantages soon appeared and they stopped it. The sum total, therefore, of results obtained with Arsenic or its salts, dating from the early observation of Livingstone, through the elaborate experiments of Bruce and Lingard, to the more recent experimental observations of Laveran, Mesnil, Thomas, and Thomas and Breinl was such as to encourage hope of a successful treatment; there was no doubt that the disease was modified, the life of many of the animals was prolonged, and some of the animals were cured. All experiments showed equally well the disadvantages of the drug, such as the recrudescence of the disease on stopping the drug and the severe toxic symptoms caused by it.

The employment of the Aniline colours

EHRLICH and SHIGA at about this time (1904) published their '*Farbentherapeutische Versuche bei Trypanosomenerkrankung*,' in the Berliner klinische Wochenschrift, 1904, Nos. 13, 14. These observers were the first to introduce colouring matters belonging to the Benzopurpurin group in the treatment of trypanosomiasis; they

obtained better results with *Trypan-red* than with either Arsenic or human serum, upon mice infected with Mal de caderas. They were able to cure mice, and in rats the parasites disappeared, but reappeared after a short time. The result of these eminent observers drawing attention to the use of Anilin colours was to stimulate a great amount of investigation in this direction.

LAVERAN and MESNIL, '*Le trypanroth dans le traitement de quelques Trypanosomiases*' (Comptes rendus de l'acad. des sciences, Vol. 139, p. 19), confirm Ehrlich and Shiga.

HALBERSTADTER (Centralbl. f. Bakt., 1905, Vol. 38, p. 525) confirms Ehrlich as regards the action of Trypan-red in Caderas, similarly in Dourine, but had very little success in Ngana.

NISSLE (Arch. f. Hygiene, 1905, Vol. 53, p. 181) found *Trypan-red* better against Ngana than against Caderas.

EWALD FRANKE, 'Therapeutische Versuche bei Trypanosomen erkrankung' (Inaugural dissertation, 1905, Jena), as the result of an extensive study of *Trypan-red* on *T. equiperdum* and Mbori strains, recommends especially the *combined treatment of Trypan-red and Arsenic.*

WENDELSTADT (Deutsche med. Wochenschrift, 1904, No. 47) found that *Trypan-red* administered in small doses internally caused the trypanosomes to disappear, but that they soon reappeared again. He was able to cure rats infected with Ngana.

WENDELSTADT (Deutsche med. Wochenschrift, 1907) by the internal application of trypan-red only succeeded in obtaining prolongation of life in the case of ngana-infected animals.

THOMAS and BREINL in a paper entitled 'Trypanosomes, Trypanosomiasis and Sleeping Sickness' (Memoir XVI, 1905, of the Liverpool School of Tropical Medicine), published a full report of treatment experiments with various drugs, amongst them *Trypan-red*, but they only succeeded in demonstrating that it helped to prolong the life of infected animals.

WENDELSTADT and FELLMER, in a paper entitled 'Uber die Einwirkung von *Brilliantgrün* auf Naganatrypanosomen' (Zeitschrift für Hygiene und Infekt. Krankheiten, Vol. LII, 1906, pp. 263-280), state that the parasites disappear from the peripheral circulation, and that after repeated doses the blood of the animals becomes negative as tested by subinoculations.

MESNIL and NICOLLE, 'Traitement des Trypanosomiases par les couleurs de benzidine' (Première partie, étude chimique. Seconde partie, étude experimentale. Annales de l'Institut Pasteur, Tome XX, June, July, 1906), tried to find out the connection between the chemical constitution and the trypanocidal action of the *benzidine colours. P. di-amido-diphenyl urea + Ac. H. (Ph.)* and *p. di-chloro-benzidine + Ac. H. (Cl.)* were the only colours which showed any marked action on the trypanosomes. Amongst the Arsenic preparations with which they experimented *Atoxyl* was found to be the most efficient.

MESNIL, NICOLLE and AUBERT (Annales de l'Institut Pasteur, January, 1907) give their experiments upon, amongst other drugs, the *benzidine colours*, and state that the blue colours are superior to the red colours, their best results being obtained with p. di-amido-diphenyl urea + Ac. H. (Ph.).

MESNIL and NICOLLE (Annales de l'Institut Pasteur, December, 1907) describe in a third paper the final results of their experiments in the case of twelve monkeys infected with *T. gambiense*, of which six were cured by Atoxyl alone, four by the combined treatment of Atoxyl and Ph., and two by Ph., first employed alone, followed up by only one injection of Atoxyl in the first case, and two in the second.

EHRLICH, in a paper entitled 'Chemotherapeutische Trypanosomenstudien' (Berl. klin. Wochenschrift, Nos. 9-12, 1907), says *Benzidine colours (Trypan-red), Triphenylmethane colours (Brilliant green* and *Malachite green*, Wendelstadt and Fellmer) have been found to be trypanocidal. Ehrlich experimented with *Parafuchsin* by feeding mice on parafuchsin cakes, which gave very good results.

WEBER and KRAUSE, in a paper entitled 'Farbstoffbehandlung der künstlichen Trypanosomeninfektion' (Berliner klinische Wochenschrift No. 7, 1907), tested systematically different colouring matters (*Crystal violet, Victoria blue, Fuchsin*), as regards their trypanocidal action in Ngana, with the view to find relations between chemical constitution and action. Fuchsin seemed to have the best effect, also because of its relative harmlessness for animals. They were unable to obtain *cures*, but the advantage of Fuchsin seems to reside in its power of prevention.

WENYON, in a paper entitled 'Action of the *colours of Benzidine* on mice infected with *Trypanosoma dimorphon*' (Journal of Hygiene,

Vol. VII, April, 1907), describes how he treated mice infected with *T. dimorphon* and 54 *Benzidine colours.* He finds, in contradiction to Mesnil, Nicolle and Aubert, that the *red* colours have a more powerful effect on *T. dimorphon* than the *blue* ones.

PLIMMER and THOMSON have used Mesnil's, Nicolle's and Aubert's *Cl. colour* (*p. di-chloro-benzidine* + *Ac. H.*) and obtained the same results as with Trypan-red.

KOCH, in a paper entitled 'Schlussbericht ü. d. Thatigkeit d. deutschen Expedition z. Erforschung der Schlafkrankheit' (Deut. med. Wochenschrift, No. 46, 1907), states that *Afridol blue* and *Afridol violet* had not the least effect upon trypanosomes, neither had *para-Fuchsin* nor *para-Rose aniline.*

C. BROWNING, 'Experimental Chemotherapy in Trypanosome infections' (Brit. Med. Jour., No. 2,446, November 16th, 1907, p. 1405), gives the results of colour treatment, especially with *para-Fuchsin.* He recommends treatment with *Atoxyl and Dye.*

YAKIMOFF, 'Zur Behandlung der Dourine' (Centralbl. f. Bakt. Orig., Vol. XLV, h. 5, Dec. '07) used *Trypan-red* for treatment of Dourine in mice, rats and rabbits. Several injections of Trypan-red are able to prevent relapses in *mice* and to effect a *cure.* During the incubation period, if Trypan-red is given very early, the appearance of trypanosomes is prevented.

It does not act prophylactically, and there is no immunity after cure. The mechanism of Trypan-red action is trypanolytic, trypanosomes being killed by Trypan-red by immune substances and other products. The immunity, however, is of very short standing.

THOMAS in his paper referred to above, and published May, 1905, states that after the publication of Ehrlich's and Shiga's results with Trypan-red, he repeated the experiments with animals infected with different species of trypanosomes. 'The best results were obtained with mal de Caderas-infected animals; the results were not so good with animals infected with nagana and surra, and still worse in the case of animals infected with dourine, the Gambian horse strain, and *T. gambiense.* The parasites disappear for a few days to reappear, and the duration of the disease was not greatly prolonged, and on analysing the evidence given above of those who have carefully experimented with the aniline dyes, we are driven to conclude that the colours do not possess any advantage over the arsenic salts, that

they are not even as efficacious.' In other words, sodium arseniate had given better results and still held out to investigators a more promising field for ultimate success than the use of colours.

Use of the combined treatment of Arsenic and colours

However, following up the experiments of Laveran, Thomas determined to try a combination of the two drugs—*Trypan-red* and *Arsenic*—and the results were more encouraging, but he states, 'unfortunately trypan-red also caused a nephritis, and by its chemiotaxic properties very extensive necrosis sometimes resulted. On monkeys, especially, the subcutaneous injection of the dye either alone or in combination with arsenious acid induced ulceration, which so undermined the health of the animals that they succumbed to any outbreak of disease, which occurred only too frequently amongst my animals. *It was these untoward accidents which induced me to seek a preparation of arsenic less toxic and the subcutaneous injection of which entailed less danger of necrosis.*' Before, however, proceeding to describe the compound of arsenic (*Atoxyl*), which he demonstrated had such a marked action upon the trypanosome, we wish to record the observations made upon the *combination of Arsenic with colours* and other bodies.

THOMAS and BREINL, in their publication referred to above, came to the conclusion:—

'That in trypan-red we possess an agent of some use in the treatment of trypanosomiasis. That certain trypanosomic diseases appear to be more amenable to its action than others. That in the substance at present available there is need for improvement in order to abolish its toxic effects.

'That a combination of arsenic and of an improved form of trypan-red would seem indicated in the further investigation of the cure of trypanosomiasis.'

LAVERAN wrote a paper entitled 'Traitement mixte des Trypanosomiases par l'acide arsenieux et le trypanroth des infections au *Trypanosoma gambiense*,' published 30th January, 1905, in the Comptes rendus de l'Academie des Sciences. He was the first to use a combination of trypan-red and arsenious acid. He made a large number of experiments upon this combination, and we owe to him most of our knowledge upon the effects of this combined treatment upon small animals. He demonstrated the curative action of the combination of trypan-red and arsenious acid in the case of rats

and mice infected with *Mbori* or *Surra*. In the case of animals infected with *T. gambiense* the results appeared to him less encouraging.

In a second paper upon the mixed treatment, published April 17th, 1905, in the Comptes rendus de l'Academie des Sciences he discusses the combined treatment of monkeys infected with *T. gambiense*. His observations confirm the results previously obtained with other animals. We reproduce his own comments upon the combined treatment in the case of *T. gambiense*.

'Il n'y a pas de motif pour que le traitement qui a reussi dans les infections expérimentales du rat, du chien et des singes par *Trypanosoma gambiense* ne reussisse pas également dans les infections naturelles, chez l'homme, et je crois que, dès maintenant on serait autorisé à essayer de ce traitement chez les sujets atteints de trypanosomiase. La difficulté sera de detérminer les doses d'acides arsénieux et de trypanroth qui devront être préscrites; des tatonnements seront inévitables. Les chances de succès seront d'autant plus grandes que la maladie sera à une période moins avancée de son évolution. Il est douteux que le traitement puisse donner encore de bons resultats quand les accidents du côté du système cérébrospinal ont acquis une certaine intensité: on se rappellera d'autre part que le trypanroth est irritant pour les reins (1), on surveillera les urines et l'on ne préscrira pas ce médicament aux malades atteints de néphrite.'

FRANKE (loc. cit.) also strongly recommended, as the result of his extensive trials, the use of the Trypan-red-arsenic treatment. WENDELSTADT and FELLMER also advocate the treatment with a combination of *Arsenic and Brilliant green*. They also combine Brilliant green and Nucleinic acid.

MAGALHAES, in a paper entitled 'De l'Action des Composés arsenicaux et du vert brilliant sur le *Tryp. gambiense* et le *Tryp. brucei*' (Arch. de. R. Inst. bact. Camera pestana, T. I, Jan. 2nd, 1907), treated rats infected with *T. gambiense* with Sodium arseniate and Brilliant green. The parasites disappeared for a time but reappeared again.

As seen from the above notes, the combined Arsenic-colour treatment led Laveran to believe that as it had succeeded in his hands

so well in the case of animals artificially inoculated with *T. gambiense*, so it might succeed in man in the case of sleeping sickness.

Employment of Atoxyl in trypanosomiasis in animals

THOMAS, however, as we have seen, had given it up on account of the nephritis and local necrosis which was induced, and set himself to find a preparation of Arsenic less toxic than Arsenious acid or Sodium arseniate, and the subcutaneous injection of which produced less danger of necrosis. An aniline compound, the anilide of metarsenious acid (*Atoxyl*), having the supposed formula $C_6H_5NH\ AsO_2$, attracted his attention. This preparation had been before the medical profession since 1900, and various workers had recorded its use in the treatment of various skin affections and in anaemia.

F. BLUMENTHAL, in a paper 'Uber Metaarsensäure anilid' (Die medizinische Woche., 14th April, 1902, No. 15), describes how he had used Atoxyl on rabbits in order to test the toxicity of the drug. He concluded from his experiments that Atoxyl was 40 times less toxic than *Solutio Fowleri*.

SCHILD (Berl. klin. Wochenschrift, 1902, No. 23, and Dermatologische Zeitschrift, Band X, Heft 1, 1903) employed Atoxyl against different skin diseases as psoriasis, lichen ruber, etc., with very good results.

F. BIRINGER published in the Therapeutische Monatshefte, August, 1903, a paper on 'Klinische Erfahrungen mit Atoxyl.' He used Atoxyl in the treatment of different skin diseases, and as a result regarded it as a valuable and welcome substitute for Arsenious acid.

MÖLLER, in a paper in the Berliner klin. therap. Wochenschrift, entitled 'Uber die Heilung der Tuberkulose mit Atoxyl,' employed Atoxyl administered intravenously in 50 cases of tuberculosis with very encouraging results.

F. MENDEL (Therapeutische Monatshefte, April, 1903), 'Zur endovenösen Application der Medikamente,' states that Atoxyl is especially suitable for either subcutaneous or intravenous administration. It produces no necrosis, no fever, and very much larger doses of arsenic can be given without producing toxic results. '*I have myself*,' wrote Thomas, '*tried the drug in high doses intravenously without ill effects.*'

To Thomas, and to Thomas and Breinl, belongs the credit of having, after a very careful experimental investigation, introduced

to the notice of the medical profession the superior claims of Atoxyl as a curative agent in cases of trypanosomiasis. Before, however, recommending it to the profession, they put it to a most searching test in animals. We quote Thomas's own words:—'Tentative experiments on rats and rabbits appeared so favourable that I decided to institute a series of experiments. These observations have been in progress for the last ten months, and have on the whole been most promising. In all my therapeutic experiments certain conditions were laid down to be followed out.

1. That the animal should be well infected and the presence of parasites determinable in its blood.

2. That the disease should have been established some time (one cannot expect to treat either man or beast suffering from the disease in the very early stages when no definite symptoms are manifested).

3. That some symptoms besides the presence of parasites should be in evidence:

(*a*) Anemia;

(*b*) Loss of weight.

'If the treatment of such infected laboratory animals be successful, then such a line of medication ought to be of service in the practical treatment of the disease.

TRYPANOSOMES

The trypanosomes experimented with are the following:—

Trypanosoma gambiense—

(*a*) Gambian fever strain (Gunjur).

(*b*) Congo Free State fever strain.

(*c*) Uganda sleeping sickness.

(*d*) Congo Free State sleeping sickness.

(*e*) A highly virulent strain derived from one of my cases of sleeping sickness which had only been passed through a monkey, baboon, and a rabbit.

Trypanosoma brucei (nagana).

Trypanosoma evansi (surra).

Trypanosoma equinum (mal de Caderas).

Trypanosoma equiperdum (dourine).

Trypanosoma dimorphum (Gambian horse disease).

ANIMALS USED

T. gambiense.—Monkeys, dogs, pups, kittens, rabbits, guinea-pigs, rats and mice.

T. evansi.—Rabbits, guinea-pigs, dogs, rats, mice.

T. brucei.—Rabbits, guinea-pigs, rats, mice.

T. equinum.—Dogs, rabbits, guinea-pigs, rats and mice.

T. equiperdum.—Pups.

T. dimorphum.—Dogs, pups, rabbits, guinea-pigs, rats and mice.'

We reproduce also his conclusions upon the advantages of Atoxyl in trypanosomiasis:—

'With five exceptions every animal has had one or more controls which were infected at the same time; the controls of *T. gambiense*, strain '*e*,' surra, nagana, caderas, and Gambian horse parasites have all died in the usual time. It is, therefore, evident, from the great majority of the experiments, that the treatment of animals infected with trypanosomes with this preparation or in combination with the dye either arrests the disease, thus prolonging the life of the animals, or apparently cures them. This is especially the case in animals infected with the ordinary strain of *T. gambiense*, and even, though to a less extent, with the abnormally virulent strain called '*e*.'

'A comparison of animals infected with the same strains, but treated according to Laveran's method with sodium arseniate has made me conclude that treatment with this aniline compound is in many ways superior to the ordinary arsenical treatment, on account of the quicker action of the drug on the parasite, the fact that the action seems to be prolonged, that large doses can be given without toxic symptoms and the entire absence from any tendency to cause sloughing.

'In my opinion treatment is indicated, of cases of trypanosomiasis in man, with this drug in high doses administered intravenously and for a long period, pushing it to the maximal amount that the case can stand without headache and nausea, at the same time building up the patient in every way possible that will conduce to a lessening of the anaemia. In the European the combination of trypan-red would probably be objectionable on account of the intense colouring of the tissues and secretions, but the native exhibits only a reddening of the conjunctivae and staining of the secretions. One of my cases of

trypanosome fever had been under treatment with trypan-red and arsenic for a short time before he returned to the Congo with favourable results—the parasites were longer absent from the peripheral circulation; his general blood condition was better; his temperature was almost normal.

'I present the results of my experiments tentatively. I have used the term "apparently cured," as any one with an intimate knowledge of *T. gambiense* and other forms of trypanosomes in animals knows how difficult it is to say that an animal is not infected. This is especially the case with the human parasite. In a previous paper I have recorded cases in which the blood has been negative for nearly a year in rats known to have been infected, and which at the expiration of that time showed parasites once more in their blood.

'I do not believe that sodium arseniate alone will be found of great practical value, nor do I think atoxyl is a perfect preparation, from its toxic effects on canines and felines, but it is an advance on arsenious acid, and, if further efforts be made to produce a substance like trypan-red, but less irritating in action, the combination ought to be of service in the treatment of trypanosomiasis in man.'

THOMAS at about the same time made a communication to the Royal Society (received April 8th, 1905, and read May 11th, 1905) upon the experimental treatment of Trypanosomiasis in animals with Atoxyl, Atoxyl and Trypan-red, and Trypan-red alone. The communication was the result of exhaustive experiments upon animals infected with five strains of *T. gambiense*, one a very virulent one taken from a case of sleeping sickness, and the rest the common animal strains. The Atoxyl was given in two ways.

(1) High doses at intervals of a week.

(2) High initial dose and then reduced amounts administered three times a week.

He concluded, '*In my hands the arsenic-anilin compound (Atoxyl) has given far better results than treatment with sodium arseniate. The advantages of its administration intravenously or subcutaneously in high doses over a length of time—namely, its less toxic properties, the absence of all tendency to cause sloughing and the apparently longer action of the drug, make me believe that the employment of this compound is indicated in the treatment of human trypanosomiasis.*'

A third paper by THOMAS in collaboration with BREINL upon

'Trypanosomes, Trypanosomiasis and Sleeping Sickness,' was published as Memoir XVI, 1905, of the Liverpool School of Tropical Medicine.

This paper contains the full report of all the treatment experiments. They wrote:—

'We have, therefore, to realise that the ordinary arsenic compounds when administered, only produce a temporary favourable effect, that, if long continued, the animals will die either from the parasite or from the arsenic, or from both. Hence, some other compound is indicated. It is for this reason that the newer compounds of arsenic have been experimented with in order to find a preparation capable of being used over a long period and in high doses without producing toxic symptoms Of the various preparations tried, a meta-arsenic anilin compound, atoxyl, has proved the most satisfactory, but it is not ideal. It is not non-toxic, as dogs, kittens, guinea-pigs, and rabbits have shewn toxic symptoms and succumbed, but it is not so toxic as sodium arseniate. It does not produce the sloughing which so often follows the subcutaneous or intravenous inoculation of sodium arseniate, it causes no pain, and its administration can be continued over a period of many months even when used in extremely high doses.

'It is the only remedy at present giving any prospects of a cure. In the treatment of cases a rational method of treatment must be adopted. It is useless, for instance, to only administer arsenic for a short period or until the parasites have apparently disappeared from the peripheral circulation. The drug must be administered in as high doses as possible, and it must be continued even until after all the favourable signs are present, viz., disappearance of parasites from the blood, increase of weight, improvement in the blood count and percentage of haemoglobin, loss of the auto-agglutination phenomenon of the blood corpuscles, decrease in and more regular temperature. From time to time susceptible animals ought to be inoculated with large quantities of the patient's blood, at least in amounts of 5·0 to 15·0 c.c. At the same time all aids in building up the physical condition of the individual should be used. If such a régime be carried out, and treatment commenced at an early period, the prognosis (based on the experience of treated animals) will be good.

'The drug was used only upon animals showing the effects of the parasites, such as loss of weight, anaemia, fever, and auto-agglutination of the corpuscles, and no animal was used until its blood contained numerous parasites. The numbers of the parasites present differed according to the species of animal and the disease. In the majority of the experiments, control animals which were not treated, and had been inoculated at the same time as the treated animals, were used. In all cases the control animal died.

'Intravenous inoculation was used only on rabbits, all other animals were injected subcutaneously. Treatment was continued for one to three months or until increase of weight, diminution of the anaemia, and entire absence of parasites from the blood, as far as microscopical examination could determine, was noted. At various periods susceptible animals were inoculated with the blood from a treated animal. When treatment had been discontinued for one to three months, or longer, the animal was bled or killed and all the blood available was used to inject susceptible animals. Inoculated animals whose blood has given negative results after three to six months, or after longer periods, have been inoculated with virulent blood, and have taken the disease, thereby showing that no immunity was conferred by the previous inoculation.

'*T. gambiense.*—Rabbit, male, weight, 2,010 grammes. Parasites appeared on the twelfth day. On the forty-sixth day numerous trypanosomes were present; it had lost weight (1,890 grammes). A blood count gave reds, 4,980,000; whites, 8,860; haemoglobin, sixty-seven per cent. For three-and-a-quarter months it received 1·0 c.c. of five per cent. solution atoxyl three times a week, gradually increasing the amount to 1·0 c.c. of ten per cent. solution. It then weighed 2,000 grammes. The blood count was reds, 6,640,000; whites, 6,200; haemoglobin, eighty-eight per cent. The blood in quantities of 10 c.c. was non-effective. The auto-agglutination of the corpuscles was lost. Thirty-two days later it was very ill; it was therefore bled to death, and the whole of its blood injected into a monkey. This monkey has never become infected. The post-mortem showed severe haemorrhagic cystitis, the bladder in parts being almost gangrenous and acute septic peritonitis, especially around the bladder. The spleen showed no congestion, but the connective tissue was slightly increased. The kidneys and liver were normal.

'Rabbit, 889, inoculated October 26: weight, 1,760 grammes. Blood count: reds, 6,620,000; whites, 6,700; haemoglobin, eighty-nine per cent. Parasites were seen from November 8 up to January 10; the trypanosomes were always present but in small numbers; they then increased to eighteen to twenty to a field. The anaemia was pronounced, and loss of weight was noted. It then weighed 1,540 grammes. Blood count: red, 3,880,000; white, 11,800; haemoglobin, sixty-three per cent. It could hardly sit up, and remained most of the time lying down. This animal was given 0·8 c.c. of five per cent. solution atoxyl. At the end of eighteen hours the parasites were absent from the blood. Doses were given twice a week, beginning with 0·5 c.c., and increasing to 2·0 c.c. of a five per cent. solution. The blood in large doses is non-infective. The animal is vivacious; the coat is smooth and thick. Average weight, 1,600 grammes. Several guinea-pigs infected for about two months, and showing twenty to forty parasites to a field, have been treated. They were injected subcutaneously with 0·3 c.c. of ten per cent. solution. At the end of the fifteenth hour no parasites were seen. Treatment was 0·1 to 0·3 c.c. of five per cent. solution three times a week for two months. The animals all increased in weight. One died sixty-two days after treatment was discontinued. Three rats inoculated with its blood never became infected. The second and third pigs were killed at the end of eighty and one hundred days after stopping treatment, and the blood used to inoculate controls. No control has shown the parasites.

'Virulent strain.—Rhesus.—Weight, 2,815 grammes. Inoculated November 16. Blood count: red, 5,220,000; white, 12,800; haemoglobin seventy-eight per cent. On November 31, parasites were seen. Two days later, twelve to seventeen to a field were noted. The weight was 2,420 grammes. Blood count: red, 4,600,000; white, 7,200; haemoglobin, seventy-three per cent. The parasites counted per mm.^{3} gave 100,000. Oedema of the eyelids and bridge of nose was present. The animal was given 0·8 c.c. of ten per cent. solution atoxyl subcutaneously. Four hours later: red, 4,450,000; white, 24,800; parasites 40,000 per mm.^{3} At the eighth hour: red, 4,740,000; white, 25,200; parasites one to eighty-nine fields. Between the fourth and eighth hours the parasites were seen to become remarkably degenerated and deformed; many phagocytes

were present. At the twenty-fourth hour after injection a count gave red, 5,050,000; white, 46,000. The blood was negative. The leucocytes remained high for a couple of days and then fell; in none of the phagocytes could any remains of trypanosomes be found. Treatment: 1·0 c.c. of ten per cent. solution was given twice a week. The animal increased in weight. The auto-agglutination of the corpuscles began to be less accentuated, and the number of erythrocytes and the haemoglobin rose. The local oedema disappeared. On the thirty-ninth day dysentery appeared, and the animal succumbed on the forty-eighth day after injection. The autopsy showed a very severe haemorrhagic and necrotic enteritis, with slightly enlarged spleen. Kidneys normal. Glands, small; inguinal group haemorrhagic. The blood was non-infective in amounts of 1·0 c.c., but infective if 15 c.c. of pure blood was used. Unfortunately, the arsenic was discontinued on the appearance of dysentery. A second monkey, inoculated from the first Rhesus just before treatment was begun, was treated with the same doses of arsenic; the parasites disappeared in the same way, but the animal quickly succumbed to dysentery.

'Many rabbits inoculated with this strain have been treated. It was found that unless treatment was started early that the majority of animals died as it was so exceedingly virulent. With these animals treatment was begun earlier and higher doses given than with the standard "Gunjur" strain. Despite treating the animals early, some died. With this strain treatment had to be kept up longer. Some rabbits have survived eight months after injection, while all the controls have died in fourteen to thirty-six days. Guinea pigs infected with this strain do not react so well to the treatment. Rats must be treated early and with high doses if treatment is to be successful. Mice infected with this strain react if treatment is commenced early enough. The action of atoxyl on the various trypanosomes has been studied, and after numerous observations, continued for the whole period during which the drug was administered, the effect appears to be as follows:—

'On administration of arsenic compounds into an animal showing numerous parasites in the blood the following action on the trypanosomes will be noticed. For the first three-and-a-half to four hours, depending on the dose used, very little change in the parasites can

be noticed. Between the fourth and fifth hour the effect on the trypanosomes is evident. Some parasites appear to be swollen and their movement is less rapid. If now a series of blood specimens be examined at intervals of twenty to thirty minutes, the following changes will be seen. The number of slowly moving trypanosomes increases, many parasites will be seen to be almost motionless. The protoplasm takes on a peculiar ground-glass appearance, and dark granules appear in the protoplasm; very often a small series of granules one behind the other, sometimes in pairs or all clumped together, are seen lying between the macronucleus and the anterior end, or distributed through the whole body of the parasite. At the same time vacuoles are observed, oftentimes very large. The trypanosomes become deformed, assuming various shapes, the most common being a kite-shaped form with fairly long flagellum, and a tadpole-like one with hardly any free flagellum. These forms especially exhibit greatly impaired movements. At the same time a noticeable increase of the leucocytes is discernible; phagocytes begin to appear, very often groups of five to seven will be seen. Up to this time (sixth to seventh hours) the trypanosomes, though decreased in numbers are still present in considerable quantities. Suddenly, in the course of an hour, the numbers may drop from forty to two to three to a field or less; coincident with this is a very marked increase in the number of leucocytes, especially phagocytes. From the ninth to the fourteenth and sixteenth hours the changes are less pronounced and rapid, the trypanosomes gradually disappear. At the eighteenth hour, provided the animal has been injected with the correct amount the parasites are absent from the peripheral circulation and, even though the blood is centrifuged, none can be found.

'From a series of these observations, we have determined that in hardly any of the forty-six continuously observed animals were parasites to be found after the eighteenth. Should, however, the drug be given in smaller amounts the process takes longer, lasting from thirty-six to forty-eight hours.

'From the experimental work with various therapeutic agents the following conclusions can be made:—

'(1) That animals suffering from trypanosome infection react

favourably to only a few agents, of which arsenic is the only drug which seems to exert a more than transient action.

'(2) That the greater the amount of arsenic introduced into the system of the animal the greater and more permanent the effect on the parasite.

'(3) That arsenic medication is indicated in the treatment of individuals suffering from trypanosomiasis. That the treatment ought to be long continued and regularly administered in as high doses as the case can stand. That all aids to building up the system should be employed.'

In a fourth paper upon 'Atoxyl in the treatment of Trypanosomiasis,' published in the British Medical Journal, Jan. 19th, 1907, by Drs. Breinl and Todd, these observers summarised our knowledge concerning the use of Atoxyl up to January, 1907. They quote Van Campenhout's private communication, in which he refers to the combination of Atoxyl and Strychnine and a cold bath, the latter for a tonic and stimulating effect. Three Europeans treated by Van Campenhout have gained weight and are apparently well. He has obtained good results by the treatment of Europeans by Atoxyl, in the first stages of trypanosomiasis. He prefers a solution of 5 per cent. rather than 10 per cent.

Todd and Breinl recommend the use of a 20 per cent. solution administered in increasing doses, up to 0·2 gramme.

The following letter was published by Professor Ross to make it clear that atoxyl was first suggested and used by Thomas and Breinl in trypanosomiasis and sleeping sickness in man, as fully established in the preceding pages.

THE TREATMENT OF TRYPANOSOMIASIS.

Sir,—Many statements having been made recently in the lay press to the effect that trypanosomiasis has been cured by various persons by the means of atoxyl, I should like to point out that this drug was first suggested and used by Drs. Thomas and Breinl, of this School, for the purpose referred to. A full account of their experiments was given in the Proceedings of the Royal Society, November 9th, 1905, vol. 76, and also in our publications, Memoir XVI. At the instance of this School large quantities of the drug have been sent to the

Congo, and several patients are now under treatment with it. The result has been an apparent success so far, but it is of course too early to speak definitely as to the final result, because it is well known that patients may survive for several years, even without treatment, and yet ultimately succumb. For example, we have had cases under observation for four years without a fatal result.—I am, etc.,

RONALD ROSS,
Professor of Tropical Medicine,
Incorporated School of Tropical Medicine.

Sept. 21*st.*, 1906.

Editor 'British Medical Journal.'

At the end of 1907 overwhelming clinical proof derived from the human subject is forthcoming, as we shall see. The statement that '*We have in Atoxyl the specific drug for trypanosomiasis, as we have in Quinine that for Malaria,*' fairly represents the view of Professor Koch.

Sir Patrick Manson, who has had the largest experience of this drug in human cases of trypanosomiasis in this country, concludes in the following words:—'The prospects of atoxyl treatment I consider most hopeful. As regards efficiency and mode of action, it seems to me that it is almost on a par with mercury in syphilis and quinine in malaria; and I think in using atoxyl we should conform our practice to what experience has taught us to be the best methods of using these other efficient and long-tried remedies. I don't believe we can kill the trypanosomes outright by one or two large doses of atoxyl, any more than we can kill the treponema of syphilis or the parasites of malaria by large doses of their respective specifics. Mercury does not immediately cure syphilis nor does quinine immediately cure malaria; but they deprive their respective parasites of their pathogenic properties and keep the patient alive and in good health till, in process of time, the parasites either die out or become permanently inert.'

By far the largest clinical experience of the drug has, however, been obtained by Professor Koch and his assistants, and their conviction is overwhelming on the advantages of this drug over all previous ones.

R. KOCH, 'Bericht über die Tathigkeit der deutschen Expedition zur Erforschung der Schlafkrankheit bis zum 25 November, 1906' (Deutsche. med. Wochenschift. Jahr. XXXIII, No. 2, 1907).

Koch treated *986 cases of sleeping sickness with Atoxyl.** *Out of 356 cases positive results were obtained in 347.* He points out the importance of the drug in early cases when it is much more efficient than in advanced cases. Koch divides his cases into two classes—'early' and 'advanced' cases. He gives a short clinical account of the symptoms. Debility, nervous excitement, trembling of the extremities, general disturbance of the psychical functions, and nervousness very often in the form of mania. In the latest stages of this disease, apathy and sleep. Atoxyl was usually administered in the forms of two double injections of 0·5 gramme. Usually, after the second injection the parasites had disappeared from the blood and from the lymph glands, but a real improvement is only to be seen three or four weeks after the injection. In advanced cases, it was impossible to drive out the parasites from the circulation, even after prolonged administration. Under the influence of Atoxyl, the trypanosomes sometimes disappeared for 30 to 40 days; when they reappeared they were only observed in very small numbers. Attempts to shorten the treatment by the administration of one large dose of Atoxyl only have given uncertain results as yet. In 12 cases, in which only one full dose of Atoxyl was given, the trypanosomes disappeared for 30 to 40 days.

R. KOCH, 'Schlussbericht über die Tatigkeit der deutschen Expedition zur Erforschung der Schlafkrankheit' (Deutsch. med. Wochenschrift, 1907, No. 46), gives his further experiences. He believes that some of the appearances, after Atoxyl treatment, can only be explained by the resorbtion of the dead trypanosomes. He still uses as the mode of administration the double injection of 0·5 gramme Atoxyl on two successive days, then 10 days interval, and two more injections. Koch was not able to observe, as Erlich did, that the trypanosomes become resistant to the Atoxyl. Attempts to administer Atoxyl in doses of one gramme were unsuccessful in so far as after such large doses of atoxyl blindness very frequently occurred, due to atrophy of the optic nerve. Very interesting from an epidemiological point of view is the fact, which he has pointed out in his previous reports, that in Kisiba fifteen married women were found suffering from sleeping sickness. As

* Up to date he has treated some 2,000 cases.

these women had never left the place, and it was found that their husbands were all either suffering from sleeping sickness or had died of that disease, Koch brings forth his own explanation: that trypanosomes may be transmitted by coitus.

Koch quotes a further case, where three women were all infected with trypanosomes from one man.

Koch states that early cases of trypanosomiasis can be definitely cured by Atoxyl by a six months' treatment; in far advanced cases it is very difficult to drive out the parasites.

BRODEN, in a paper entitled 'Les Trypanosomes dans l'Etat du Congo' (Rapport sur les traveaux du laboratoire medicale de Léopoldville de 1900 à 1905, II, Bruxelles, Hayem, 1906, p. 71-143), used Atoxyl in the treatment of one case of trypanosomiasis with very encouraging results.

BRODEN ET RODHAIN 'Le traitement de la trypanosomiase humaine (Maladie du Sommeil)' (Archiv für Schiffs und Tropenhygiene, Band X, 1906, p. 693), treated cases of trypanosomiasis (three white men) with Atoxyl. Fever disappeared in all three and their general heath improved.

VAN CAMPENHOUT starts with 0·2 gramme of Atoxyl, and increases his doses 0·05 gramme every second day till he reaches 0·8 gramme. This does is given every second day for a fortnight or three weeks, and then he decreases his doses gradually 0·05 gramme. In the cases of the second period he uses strychnine and cold baths.

MESNIL, NICOLLE ET AUBERT, 'Recherches sur le traitement des infections experimentales à *Trypanosoma gambiense*' (Annales de l'institut Pasteur, Tome XXI, Jan., 1907, pp. 1-19), continue the researches on a number of Benzidine colours and Sodium arseniate and Atoxyl on *Trypanosoma gambiense*. Amongst the Benzidine colours in general, the blue colours show themselves superior to red colours. The best results were obtained with p. di-amido-di-phenyl-urea + AcH (Ph.). Atoxyl is considered superior to Sodium arseniate. Further, they combined the colour Ph. with Atoxyl with very good results.

KOPKE publishes his experiences in the following papers:—

'Trypanosomiases humaines,' (XV Congrés international de médecine de Lisbonne). 'Traitement de la maladie du Sommeil,' (Travaux de l'École de Médecine Tropicale de Lisbonne). 'Traite-

ment de la maladie du Sommeil,' (Rapport presenté au XIV Congrés Intern. d'Hygiene et Demographie. Lisbonne, 1907).

His results with atoxyl are not very good. Out of 29 cases, 22 died from trypanosomes. Seven are still alive and of these only two are in good health.

Kopke used very large doses of Atoxyl (one gramme in one injection). Out of 29 cases, six had eye lesions which were diagnosed as atrophy of the optic nerve, and were certainly due to the over-doses of atoxyl given.

THIROUX ET D'ANFREVILLE, 'La maladie du Sommeil au Senegal,' record, 'trois cas traités, guérison dans un cas.' Rapport de Laveran, (Bull. Acad. Méd. Séance de 26 fev. 1907).

HOLLEBEKE, 'Traitement de la trypanosomiase par l'atoxyl. Notes cliniques et therapeutiques,' (Bull. acad. roy. de méd. de Belgique, Vol. XXI, 1907), treated eight Europeans for trypanosomiasis. Gave daily injections of 0·2 gramme of Atoxyl. Never observed any eye-symptoms. After six months, he stopped the treatment and the same improvement continues. He considers his cases as cured.

JAKIMOFF, 'Zur Atoxylbehandlung der experimentellen Dourine,' (Deutsche. med. Wochenschrift. 1907. No. 16), cured white rats infected with Dourine by Atoxyl.

MARTIN, in a paper entitled 'Maladie du Sommeil. Cinq nouveaux cas de trypanosomiase chez les blancs. Essais de traitement,' (Ann. de Inst. Past. Tome XXI, Mars, 1907), states that Atoxyl has a very noticeable action on the trypanosomes, which disappear from the blood, as in malaria the parasites disappear under quinine treatment. Concerning whether or not a permanent cure has been effected, he states that it is impossible to say anything as the time was too short.

COOK, at a meeting of the Society of Tropical Medicine and Hygiene, Oct. 26, 1907, discussed the question of sleeping sickness in Uganda, and stated that Atoxyl was by far the best drug for the cure of the disease that had yet been tried, and 'that it was greatly to the credit of English investigators that an Englishman, Thomas, of Liverpool, first applied it to the treatment of sleeping sickness.'

UHLENHUTH, 'Demonstration von mit Atoxyl behandelten Dourinekaninchen' (Deut. med. Wochenschrift, 1907, No. 30);

UHLENHUTH, HUBENER and WOITHE, 'Experimentelle Untersuchungen über Dourine mit besonderer Berucksichtigung der Atoxyl behandlung' (Arb. a. d. Kaiserl. Gesundheitsamte, Bd. XXVII, h. 2, 1907);

UHLENHUTH, GROSS and BICKEL, 'Untersuchungen über die Wirkung des Atoxyls auf Trypanosomen und Spirochäten' (Deutsche med. Wochenschrift, 1907, No. 4), give the results of treatment of experimental animals infected with *T. equiperdum* (Dourine) with Atoxyl (*a*) preventive, (*b*) curative. Preventive results were not very encouraging. They were able to drive the Dourine parasites out of rabbits, rats and mice, and to keep the animals alive. The authors do not state whether it is a definite cure, as the time of observation was too short.

Ehrlich's experiences with Atoxyl in experimental treatment are very favourable. He was able to prove that the different trypanosome strains become after a time resistant to the drugs, and he got the 'festen' strains; an Atoxyl resistant, a para-Fuchsin resistant strain, which did not even in sub-inoculation react to the drug.

The Atoxyl resistance may partly explain the unfavourable results in some cases of sleeping sickness, as described by Kopke and Broden.

To FOURNEAU (Journal Phar, et Chim., 6ième série, T. XXV, 1 April, 1907) is due the credit of showing that, chemically, atoxyl is not a new preparation, having been synthetized as long ago as 1863 by Bechamp in the early days of the synthesis of aniline colours, fuchsin being produced in abundance at the same time. Bechamp supposed that he had in hand an anilide of ortho-arsenic acid. Fourneau supports this view, and the body is described as an anilide, but this from the evidence given by Moore, Nierenstein and Todd, and by Ehrlich and Betheim, is probably an error.

EHRLICH, in a lecture delivered before the Berliner medizinischen Gesellschaft, February 13th, 1907 (Berl. klin. Woch. No. 9-12, 1907), stated that Atoxyl was the sodium salt of p-amido-phenyl-arsenic acid, with four molecules of water of crystallization. The analyses of MOORE, NIERENSTEIN and TODD (Bio-Chemical Journal, Vol. II, Nos. 5-6, 1907, pp. 300-374) yield the formula (NH_2) (C_6H_4) AsO, $ONaOH,3H_2O$, and they had independently come to the conclusion that the arsenic radicle was united directly to the ring. Since then

Ehrlich and Bertheim have published the details of their chemical work, showing that the arsenic radicle is in the para position to the NH_2 group.

Moore, Nierenstein and Todd show that Atoxyl is not, as it was originally described, an anilide of metarsenious acid, but is an exceedingly stable chemical substance with the arsenical radicle directly attached to the benzene ring. It was shown that the aqueous solution is strongly dissociated electrolytically, giving in consequence an apparently low molecular weight by the freezing point method and possessing a high electrical conductivity. Except on standing in aqueous solution, it is a most stable compound, and neither aniline nor arsenic are easily detachable from its molecule by chemical means.

Its toxic properties are neither those of arsenic nor of aniline even when pushed to excess, and its therapeutic action is rapid; from this and its high conductivity, showing high dissociation, the conclusion was drawn that its activity must be ascribed, not to free inorganic ions or to free aniline, but to a complex organic ion containing both the arsenical and aniline radicles.

Since the introduction of Atoxyl for the treatment of trypanosomiasis a large number of observers have tested it, and all are now united in giving it the premier position as a trypanocide.

The results of most observers upon the treatment of sleeping sickness by Atoxyl alone completely confirm the results stated above as having been obtained by Thomas and Breinl in the experimental trypanosomiasis of animals. Thus, cases have been described which were apparently permanently benefited and might be described as cured, but in a great many of them recurrences were observed, and finally the infection became persistent, the trypanosomes becoming 'Atoxyl-fast' and being apparently no longer affected by the drug.

Quite recently a second distinct advance has been made in the experimental therapeutics of trypanosomiases by workers of the Liverpool School. This consists in treating infected animals, from which the trypanosomes have primarily been driven out of the blood by the use of Atoxyl, by a second drug, so as to prevent the recurrences which so often follow Atoxyl treatment alone.

The general principle underlying the combined method of treatment by two successive and quite different drugs is that when an

infective organism, such as a protozoon, shows two distinct phases in its life-history, then these two phases ought to be attacked by separate drugs, and it is not only possible but probable that a drug which affects the first will not affect the second, and *vice versâ*.

The application of this bio-chemical principle, which has led to success in the prevention of recurrences in trypanosomiases, may be very wide in experimental therapeutics, especially in protozoon diseases where different phases in the life-history occur in nearly all cases. The two drugs necessary to attack two successive stages of the parasite will certainly not always be found to be an arsenical compound and a mercurial compound; but it is established as a principle that given a parasite has two successive phases, A and B, then the problem of experimental therapeutics is to find two remedial agents, *a* and *b*, of which *a* kills phase A and *b* kills phase B, and then to apply the remedies *a* and *b* in succession, killing off phase A as completely as possible with remedy *a*, and then attacking phase B with remedy *b*, and continuing this rotation until the animal is free of infection.

The research on the combined treatment was commenced in the Bio-chemical Laboratory of the University of Liverpool by B. Moore, Nierenstein and Todd in October, 1906, and a preliminary report published in March, 1907, followed by a fuller account in May, 1907; the work is now being continued on large animals in the Runcorn Laboratory, and, as far as cases are available, upon human trypanosomiasis.

At the time of the commencement of this research, a second phase in the life cycle of the trypanosome was not known with certainty to exist, but since then the life-history has been more completely investigated by J. E. Salvin-Moore and Breinl, and these observers have clearly shown the existence of such a phase.

The existence of this other phase was suspected by Thomas and Breinl, Moore, Nierenstein and Todd, from the regular way in which, after the trypanosomes had been completely driven out of the peripheral blood by Atoxyl, recurrence again took place, although the investigation of organs and tissues other than the blood had failed to demonstrate anywhere a storage of the ordinary phase of trypanosome.

On these grounds, these observers determined to first drive out

the parasite from the peripheral blood by Atoxyl, and then when the blood was free to treat by other drugs, paying no attention to whether these drugs had any effect upon the usual phase of the trypanosome or not.

Accordingly a series of experiments was commenced, in which rats infected experimentally with *Trypanosoma brucei* were used on account of the rapidity of action of this type of trypanosome. After driving out the trypanosome with Atoxyl, the salts of the different heavy metals were given; salts of Silver, Lead, Copper and Antimony respectively were employed without any marked results, but when Mercury was used in the form of the bichloride a distinct beneficial result was at once obtained. While the entire series of controlled rats treated with Atoxyl alone succumbed, nearly 70 per cent. of the rats given the double treatment survived, never showing any recurrence of trypanosomes, and of the remaining 30 per cent. only 8 per cent. showed recurrences of trypanosomes.

It may be emphasized that the Mercury salt alone has not the slightest effect upon the ordinary phase of the trypanosome as seen in the peripheral circulation. This appears to demonstrate clearly that the two drugs act upon two quite distinct phases.

Similar results are at present being obtained with other classes of animals, and indications of like results with the more slowly acting *Trypanosoma gambiense* of sleeping sickness.

The two drugs are likewise being employed in the treatment of sleeping sickness in man, and the results so far obtained are distinctly encouraging.

PLIMMER and THOMSON, 'A Preliminary Summary of the Results of the Experimental Treatment of Trypanosomiasis in Rats' (Proc. Roy. Soc., July 20th, 1907), got the best results by combining Atoxyl and the different Mercury preparations (Sozoiodol, Donovan's solution), and certainly some of the rats were cured. They also used Iodipine. Plimmer and Thomson were able to confirm Ehrlich's experiments on atoxyl-resistance.

Antimony

PLIMMER and THOMSON, 'Further Results of the Experimental Treatment of Trypanosomiasis in Rats' (Proc. of the Royal Soc., read Nov. 7th, 1907), recommend for the treatment of sleeping sickness Potassium antimonyl tartrate. Trypanosomes disappear very rapidly, but there is a bad effect on the rats treated. They

suggest the use of Sodium antimonyl tartrate. Out of 25 rats which have been treated with this substance, 23 had no relapses from 25 to 26 days. No local disturbances are caused by the injection of the drug into rats, in doses of up to 0·5 centigramme.

In the hands of one of us (A. B.) Sodium antimonyl tartrate has not given the good results Plimmer and Thomson described. A fairly virulent strain of *T. equiperdum* was used. Out of twelve rats treated with two to four injections of 0·25 c.c. of a 1 per cent. solution (0·35 of the same solution was the fatal dose) only one rat which was treated during the incubation period of the disease is still alive, all the others having died ten to fifteen days after the last injection of the drug, their blood swarming with parasites. One horse infected with a strain of cattle trypanosomes, brought back from the Congo, has been under treatment since December 9th, having received five intramuscular injections, 10 c.c. of a 1 per cent. solution of Sodium antimonyl tartrate. It is still showing parasites in a very small number from time to time; they were seen even three days after the last injection. The local effects of the drug in rats are very severe: necroses and sloughing.

The parasites, however, disappear very rapidly indeed, generally speaking more so than after a corresponding injection of Atoxyl.

But notwithstanding our results, we consider that the introduction of another metal (Antimony) belonging to the same group as Arsenic is a further progressive step in the treatment of sleeping sickness, and is moreover very suggestive.

MY EXPERIENCE OF TRYPANOSOMIASIS IN EUROPEANS AND ITS TREATMENT BY ATOXYL AND OTHER DRUGS

BY

SIR PATRICK MANSON, K.C.M.G., F.R.S., &c.

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In view of the recently recorded experiences of Campenhout, Broden, Kopke, Koch and others in the treatment of human trypanosomiasis, especially in negroes, by atoxyl, it may not be inopportune if I gave some account of my experience of this disease in Europeans, and of atoxyl and other drugs in its treatment.

My experience of trypanosomiasis in man extends to seventeen cases—seven negroes, ten whites. The negroes, who had been brought to Europe for purposes of clinical study, and because they had already entered on the terminal phase—sleeping sickness—of the infection, all died. They did not have the benefit of Thomas's important discovery of the therapeutic value of atoxyl. I shall not allude to them further.

Of the ten whites, three of the cases have been recorded already by myself and others. For the sake of completeness I shall briefly mention here these three cases, along with the seven unrecorded cases, giving them along with the latter in the order in which they came under my observation, but referring the reader to the medical journals for details.

I.—Mrs. H. M. was first seen by me on July 17th, 1901. She was then 40 years of age, and had resided on the Congo for two periods of two years and one year respectively. During the latter period she had suffered much from fever. Being pregnant at the time she came home, arriving in England in April, 1901. She had fever all the way home. A week after her arrival her child was born, and from that time till the date of her visit to me she had attacks of fever lasting for three days at a time and occurring at intervals of seven days with considerable regularity. She also suffered with pains in her

hands, ankles and knees, for which she took sodium salicylate with some relief. She informed me that the attacks of fever were preceded and accompanied by a circinate erythematous eruption on her face, limbs and trunk, and that this tended to subside with the subsidence of the fever. She was anaemic and her skin showed traces of the erythema she referred to. The spleen and liver were not palpably enlarged.

As the trypanosoma had not been discovered at that time I regarded the case as one of malaria, and prescribed a systematic course of quinine.

I saw this lady again on April 9th, 1902. She told me she had improved, notwithstanding the fatigue consequent on the illness of her baby which had died on December 19th, 1901 (? trypanosomiasis). Three weeks before her visit to me she had caught a chill and the fever, which had been in abeyance for a long time, had returned. During this period of three weeks she had had three attacks. Nevertheless, as compared with her condition in July, 1901, she had put on flesh and no longer appeared anaemic. She informed me that the patches of erythema still showed themselves at times, but were less pronounced than formerly.

I did not see this patient again at this time, but learned that she had an attack of irido-cyclitis and that subsequently she returned to the Congo.

In the autumn of 1902, after having learned from Dutton's case and from Case No. 3 (to be presently alluded to) that irregular fever along with erythema multiforme were in a patient from tropical Africa probably symptomatic of trypanosomiasis, I wrote to her husband on the Congo requesting that an examination of this lady's blood be made for trypanosomes. Before receiving a reply to my letter I heard from Dr. Broden that he had examined her blood and had found the parasite.

Dr. Broden put her on arsenic (Fowler's solution). The case did well. Fever and trypanosomes disappeared. When I saw her during a subsequent visit to England in 1906-7, by way of encouraging them, I showed this lady to two trypanosomiasis patients I had at the time under treatment as an example of recovery from the disease. She was stout and healthy looking, and was free from all symptoms of the infection. I hear she is still in excellent health.

This patient must have received her infection some time during 1900. The conclusions that she has overcome the infection and that trypanosomiasis in man is not necessarily fatal are, it seems to me, justifiable.

Dr. Broden has published his notes of this case.

II.—H. K. This was Forde's original case in which *Trypanosoma gambiense* was first definitely recognised (and for the first time in human pathology) by Dutton. It has been fully described by Dutton in the publications of the Liverpool School of Tropical Medicine, and elsewhere by Forde. I mention it here as it constituted my first conscious experience of trypanosomiasis in man. I saw the case in August, 1902, and had the clinical points of the disease demonstrated to me by Dutton. It was from what I saw on that occasion that I was enabled to recognise clinically the disease in the next case. The medicinal treatment consisted principally in the administration of arsenic, quinine and urotropin. The patient died the following January, about one year and eight months after the presumed date of infection.

III.—Mrs. S. was seen by me for the first time in October, 1902. She presented the usual clinical picture of trypanosomiasis, and the parasite was found in her blood. The case has already been fully recorded in the British Medical Journal of May 30th and December 6th, 1903, and elsewhere. I may mention here that the first indication of the disease occurred in August, 1901, supervening, apparently, on an insect bite on the leg. The patient died of sleeping sickness on November 26th, 1903, two years and three months after infection. The treatment included arsenic, quinine, methylene blue, and many other drugs, but not atoxyl.

IV.—W. Z., an engineer on one of the lake steamers in Uganda, came under my observation on October 9th, 1905. His story was that early in the year he broke his leg; that on this account and because he suffered from fever he had been in hospital in Uganda for a considerable time; and that trypanosomes having been found in his blood he was invalided on July 26th. On arrival he went to his home in Scotland, where, with the exception of two days' fever, he kept well and put on flesh. He stated, however, that he had suffered from dull pains in his legs and that once he had a swelling in his left foot.

When I saw him, there was a well-marked circinate erythema on his chest, and the cervical and inguinal lymphatic glands were enlarged. On examination of the blood trypanosomes were found. His pulse was rapid—108—his spleen palpable, knee jerks exaggerated, slight right ankle clonus. He complained that he felt weak, and also of subjective symptoms of numbness in the legs. Otherwise he appeared to be well. Blood count 4,000,000. He was sent to hospital.

During his stay there he had a malarial attack and benign tertian parasites were found in his blood. Quinine quickly got rid of this infection. He also developed a specific periostitis which yielded to potassium iodide.

From October 18th, 1905, to January 25th, 1906, he was treated by trypanroth and, later, by trypanroth and arsenious acid, the latter hypodermically. The erythema persisted, however, or, if it faded for a time, would again return. Occasionally the erythematous spots gave one the impression that they were slightly oedematous. Trypanosomes were also occasionally found in the blood, but gland puncture, which was twice practised, was negative. The temperature, except during the malarial attack, remained normal throughout.

The skin and urine became deeply stained by the trypanroth. On January 26th, albumin having appeared in the urine, the trypanroth and arsenious acid were stopped. On February 5th treatment by atoxyl injections was commenced. Beginning with one grain twice a week it was gradually raised to four grains twice a week, at which dose it was continued till the spring of 1907.

Very soon after commencing the atoxyl he became conscious of an improvement in his general health. The erythema no longer showed itself on the trunk, and for over six weeks, notwithstanding frequent and prolonged search, trypanosomes could not be found in his blood. He now insisted on leaving hospital, promising to continue treatment and to report from time to time.

He returned to his home, where he kept in perfect health and gained weight. Wishing to get reinstated in his former appointment in Uganda, he came to London during the autumn of 1906. His blood was again carefully examined for trypanosomes, but none were found. However, a monkey injected (25th August) with his blood developed trypanosomiasis and died.

Once more he returned to Scotland and continued the injections, his health keeping satisfactory in every respect. Early in 1907 he again came up to London. Again his blood was carefully examined with negative result. A monkey injected with the blood failed to develop trypanosomiasis. It was not considered prudent to allow him to return to Uganda, but he was encouraged to seek employment elsewhere, and when an appointment offered in the West Indies he left England in the early spring of 1907. On the voyage out he was shipwrecked, and during eleven days suffered great hardships, wandering about the mangrove swamps at the mouth of the Magdalena river. Nevertheless he kept in perfect health, and when I saw him on his return on 19th April, 1907, he appeared healthy and robust, without trypanosomes in his blood and with no sign of trypanosomiasis about him. As he had lost his syringe and atoxyl when shipwrecked, he had had no injections for many weeks.

Subsequently he again set out for the West Indies, and when last heard of was still in perfect health.

V.—J. M., a botanist and agricultural expert, was in British Central Africa from June, 1897, to September, 1899. I examined and passed him for service in Uganda in March, 1901. He was then 30 years of age and in good health.

He arrived in Uganda on June 17th, 1901. He informed me that before crossing the lake he had several small fevers (probably malarial) at Kusumu. He also informed me that a fortnight after his arrival in Etebbe he was bitten by 'something' in front of his left ankle; the part swelled and he had fever about the same time. With this exception he kept well for a year. About June, 1902, he began to ail—languor, loss of appetite. At Christmas of that year, and on and off till March of 1903, he had several heavy fevers, accompanied by cerebral symptoms. He became melancholic, had delusions of persecution, and at one time was suicidal. Trypanosomes were found in his blood and he was invalided home.

I saw him on August 1st, 1903. He had no fever then, and looked fairly healthy, but his manner was strange and there were definite trypanosoma erythema patches on his trunk and I found trypanosomes in his blood.

I saw him subsequently from time to time. Occasionally he had

puffy erythematous patches on his face and trunk, and once a big, swollen red and very tender patch on one gluteal region. Occasionally he had slight fever, and trypanosomes could usually be found in his blood. A principal complaint was of weakness and stiffness of the legs.

He was subjected to a variety of treatments, including arsenic and trypanroth, but not, so far as I know, atoxyl.

I lost sight of him. I heard that ultimately well marked sleeping sickness symptoms set in, and he died in University College Hospital in 1906.

VI.—Mrs. R., aged 31, had resided on the Congo (Bogandango) from 1898 to 1902, and, with the exception of fever for three days, during all that time had enjoyed good health. After furlough in England she returned to the Congo in September, 1903. She kept fairly well till June 15th, 1905, when she had a severe attack of abdominal pain, vomiting and diarrhoea. These symptoms recurred three days later (June 19th), when they were accompanied by fever and, on the following day, by haemoglobinuria. The haemoglobinuria persisted for five days. During convalescence she noticed that her right ankle had become enormously swollen, purplish in colour and very painful; at the same time a gland in the corresponding groin enlarged to the size of a hen's egg. Fever returned on June 26th and subsequently, off and on, about every four or five days, till September, when, being greatly debilitated, she was ordered home. On her way down river she was examined by Dr. Broden at Leopoldville, who found trypanosomes in her blood, besides enlarged cervical glands and a rapid pulse (120) with a normal temperature.

I saw Mrs. R. on October 9th, 1905. She was then very much emaciated, feeble and sallow. There were patches of ringed erythema on her chest and flanks and some enlarged cervical glands (right supra-clavicular). The spleen also was enlarged, and over the right ankle I found some reddish staining of skin, the remains of the inflammation in June. Trypanosomes were present in the blood; blood count 3,700,000.

She was sent to hospital and treated with arsenic and, later, Donovan's solution. On November 22nd she commenced atoxyl injections up to two grains twice a week. Over that dose the drug

seemed to cause nausea. Relatively the dose was a medium one, as she weighed only ninety pounds when treatment commenced.

On November 1st her right ankle became swollen and so painful she could not walk; but from the time atoxyl was commenced improvement set in and persisted. The erythema and the trypanosomes disappeared; temperature, which had been variable, became steady and normal, and her weight rapidly increased. Within a couple of months, from being sallow and emaciated she became ruddy and plump, and felt in excellent health.

After her discharge she returned from time to time to hospital to show herself and to have her blood examined. Trypanosomes were not found again; the erythema did not return; monkeys injected (January 12th, 1906) with her blood were not infected (March 29th, 1906), and good health continued. She left for the Congo on 8th March, 1907, against advice, but promising to continue the atoxyl injections.

I am informed that a letter, dated 11th September, 1907, had been received from her husband in which it is stated that 'she has been getting stronger and has been able to do quite a lot of language work. But she has to take great care, for evidently there is something causing a rise of temperature occasionally.' Soon after her arrival on the Congo she had a haemorrhage of some sort which pulled her down very much; apparently, she has taken some time to recover from this; possibly, judging by the temperature, the trypanosomes are again active.

VII.—H. C. C. S., aged 36, an engineer, arrived at Benguella, Portuguese West Africa, in June, 1904. His work took him up country some 100 to 150 miles from the coast. He had his first fever the following November. Attacks recurring very frequently, he had to be invalided, and arrived in England on May 29th, 1905. Soon after landing he had two attacks. I first saw him on June 23rd. He had no fever at the time, but he was anaemic and his spleen was enormously enlarged. I put him on quinine, 15 grains every tenth and eleventh day. Notwithstanding these doses fever kept recurring every few days. My *locum tenens* saw him on August 15th and ordered him five grains of quinine three times a day, apparently with benefit, for in a note dated August 24th it is stated that he had no fever, and that the spleen could no longer be felt. Subsequently

the patient married and returned to Benguella during the autumn of 1905.

For a time he kept fairly well and did much hard work in the interior on railway construction. He remembers that about this time he was bitten or stung on the leg by some unrecognised animal, supposed to be a centipede or scorpion. The part swelled and was very painful.

Fever now returned and, in addition, he got dysentery. Between the two he became so ill that he had once more to return to England, where he arrived on February 17th, 1906.

I saw him the same day. He was in bed. He had no fever at the time, but he was intensely anaemic, emaciated and weak, and evidently very ill. Both spleen and liver were much enlarged, the latter being tender as well. He was passing from four to five dysenteric stools daily. Quinine he said, aggravated the dysentery and gave him severe gastralgia. I examined his finger blood but found no malaria or other parasites in it. As he had not taken quinine recently, I was surprised at the absence of malaria parasites. I saw him daily, and on 22nd February, observing that the spleen had undergone a sudden increase in size, and from this suspecting an impending malarial attack, I gave orders that I should be sent for so soon as temperature rose. A few hours later I was summoned. The temperature was then 103°. I took blood films and found in them, not malaria parasites, as I expected, but considerable numbers of trypanosomes. I now made a careful examination of the skin and lymphatics, and recognised several characteristic patches of erythema and at least one definitely enlarged posterior cervical gland. Temperature rose on this occasion to 106° but quickly fell, although for three or four days some fever of a remittent type persisted. Trypanosomes were found for some days, their numbers gradually becoming fewer under the atoxyl treatment which was at once instituted.

From time to time, at intervals of a few weeks, although the general condition of the patient, including the dysentery, steadily improved, there were short recurrences of fever, each recurrence being associated either with the appearance of trypanosomes, or of benign tertian malaria parasites in the blood; so that without the microscope it was impossible to say whether a given relapse was trypanosomal

or malarial in nature. On the discovery of the malarial infection quinine was given systematically at definite intervals as well as atoxyl, and was now very well borne.

At first the atoxyl was given in one grain doses (10 per cent. solution) hypodermically every third day. It was gradually raised to 2·3 grains, beyond which, after not a few attempts, it was impossible to push it. Every time a 2·5 grain dose was given, violent and alarming gastralgia ensued. The 2·3 grain doses of atoxyl, and occasional 10 grain doses of quinine were therefore steadily persisted with.

Under the persevering use of these drugs and with careful nursing the patient slowly improved, the erythema, the adenitis and the dysentery disappearing. When the weather became milder, and he could walk about, the patient was removed to a healthy and bracing place in the country, where I saw him from time to time. There the febrile attacks became milder, returning at longer intervals, the trypanosomes being found only occasionally in the blood and never in large numbers. In the course of the summer of 1906 he suffered at one time from severe dental neuralgia, and twice from smart attacks of orchitis. Notwithstanding this, general improvement continued. He spent the winter of 1906-7 on the high Alps, where, with the exception of a brief but painful attack of what might have been erythema nodosum in one leg, he kept quite well and gained strength. I saw him again on July 25th, 1907, and noted that 'he had had no fever since 21st February with the exception of that attending a slight cold in May (temperature 101°). Weight 9½ stone—the highest he has ever been in his life. Feels quite well.'

He refused to consider himself any longer an invalid. A situation was offered to him in South America, my consent being a condition of the appointment. This I promised provided injection of his blood into monkey, rat and guinea-pig proved negative. These injections were made in August. All the animals were alive and free from trypanosomes in October when he sailed for South America in the best of health, promising to keep up the atoxyl injections for another year and to report progress.

A letter just received and dated 31st December, 1907, stated that 'he is very well indeed,' and 'to see him now no one would think he had ever had an illness.'

VIII.—C. G., aged 34, an engineer, was stationed at Katanga on the Upper Congo (Lualaba river) for three and a half years. He took five grains of quinine daily and enjoyed excellent health till November, 1906. About that date he began to suffer from fever, apparently uncontrolled by quinine. On November 9th, his temperature at the time being 102°, he started to return to England viâ Rhodesia and the Cape, riding 500 miles of the way on a bicycle and suffering from fever all the way. On the voyage from the Cape he gave up taking quinine as it seemed to do him no good. Fever persisting, he became bilious and yellow.

I saw him on his arrival (January 5th, 1907). His temperature was 103°, pulse 112. He was somewhat emaciated, slightly icteric and markedly anaemic. Liver and spleen, especially the latter, were both enlarged. The superficial cervical glands were also slightly enlarged and the skin of the trunk was splashed with rings and patches of erythema multiforme. A blood examination gave 2,848,000 erythrocytes per c.mm. and 4,000 leucocytes per c.mm., the large mononuclears being in marked excess (37·3 per cent). Malaria parasites were not found at that time, but trypanosomes were numerous.

Atoxyl.—He was at once put to bed and atoxyl in hypodermic injections begun. At first the injections were given every second day, the dose being rapidly increased from $1\frac{1}{2}$ to 7 grains, and occasionally 8 grains. In a few days trypanosomes had disappeared from the peripheral circulation, so that when Dr. Todd, of Liverpool, saw the patient with me on January 11th he failed to find a single specimen during a prolonged examination. The skin eruption, the adenitis, the fever and, in great measure, the debility disappeared equally rapidly. On January 13th and 14th there was a return of fever which, on microscopical examination, proved to be malarial—benign tertian. Quinine was now given in addition to the atoxyl and repeated at intervals ever since.

Except during four short periods the atoxyl injections have been continued. They were intermitted from May 26th to June 12th, from June 26th to July 9th, and from July 16th to July 25th, when he was taking perchloride of mercury; and also from November 6th to November 27th (three weeks), when he was being treated with sodio-tartrate of antimony. The dose of atoxyl varied from $2\frac{1}{2}$ to

9 grains; latterly it has been 3 grains every second day. At no time has there been any local reaction or sign of arsenical poisoning.

Perchloride of mercury.—Notwithstanding several febrile attacks and frequent and careful examination of the blood, no trypanosomes appeared in the peripheral blood till May 21st. On that day a few were found. Atoxyl in 8-grain doses was being administered at the time. The same dose was repeated on the 22nd, 24th, 26th and 28th. The trypanosomes having disappeared, hypodermics of perchloride of mercury in $\frac{1}{2}$ per cent. solution were commenced and repeated daily for fourteen days, the dose being rapidly increased from 15 to 30 minims (about one-seventh of a grain), at which it was kept for five days, when atoxyl was resumed in $2\frac{1}{2}$-grain doses every second day. A week later there was another febrile attack concurrent with a fresh invasion of trypanosomes. The dose of atoxyl was now increased to five grains. After four injections of this strength, and the trypanosomes having disappeared, the perchloride was resumed from June 27th to July 6th. On July 10th there was a rise of temperature to 101·6°, but trypanosomes were not found, though carefully searched for; the atoxyl was again resumed. The perchloride injections having caused much pain and irritation, Hydrarg. c. creta, one grain three times a day, was substituted and continued from the 15th to the 22nd of July, when, the gums being slightly tender, it was stopped and the atoxyl resumed. On August 11th temperature rose to 101·7°, and trypanosomes once more appeared in the blood.

Parafuchsin.—Treatment with parafuchsin (kindly suggested and supplied by Professor Ehrlich) was begun on August 16th, atoxyl in $2\frac{1}{2}$, $3\frac{1}{2}$ and occasionally in 8-grain doses every second day being continued at the same time. Beginning with 5 grains, the dose of parafuchsin was gradually raised by 5 grains at a time till 20 grains were taken three times a day by the mouth in cachet. These large doses, though continued till October 13th (nearly two months), caused no disturbance. There was no intestinal irritation, nor, although the urine and sweat were coloured by the drug, was there albuminuria or urinary irritation. Trypanosomes, which were present when the parafuchsin was commenced, persisted till August 29th—a fortnight. After this, though looked for almost daily, they disappeared for a time.

The last dose of parafuchsin was taken on October 17th. Ten days later there was a rise of temperature, and once more the parasites appeared. About this time the patient apparently got a chill while salmon fishing and there was some rise of temperature, which was repeated on November 1st and 2nd, when a very large influx of trypanosomes was noted.

Sodio-tartrate of antimony.—On November 8th I administered a hypodermic injection of sodio-tartrate of antimony (kindly supplied by Mr. Plimmer)—$\frac{1}{2}$ a grain, and followed it up with injections of 1, $1\frac{1}{2}$, 2, and 2 grains on the 10th, 11th, 12th and 15th respectively. There was no nausea, intestinal disturbance, or albuminuria following the injections, but the local pain and irritation were great. The parts became puffy, then hard, then fluctuating. One swelling, which had become very tense and discoloured, and seemed as if about to rupture, I incised. About an ounce of dark sanious fluid escaped, and after a week an ash-grey slough was seen at the bottom of the wound. The slough took a fortnight to come away, and the wound, which never suppurated, took a very long time to heal. The other swellings took several weeks to subside. The pain consequent on the injections was very great, demanding morphia. It would subside towards morning and be comparatively in abeyance during the forenoon, but each afternoon it would wake up again, being most severe during the night, rendering sleep impossible. There was very little rise of temperature. The pain consequent on an injection lasted about a fortnight.

It was remarked both in this and in another case that although given in different strengths ($\frac{1}{2}$ to 1 per cent.) the weaker solution of antimony was quite as painful as the stronger; and, also, that intramuscular injection, though equally painful at first, caused less swelling than subcutaneous injection.

When it became evident that hypodermic injection of antimony was impracticable, the drug was given by mouth to the extent of two grains a day diluted in two or three pints of water. This, along with three grain doses of atoxyl, is being continued. It causes no nausea or irritation of any sort.

Up to the date of this note there has been no return of fever. The patient is leading an active country life and is feeling perfectly well.

IX.—W. R. E., aged 46, was in the West Indies from 1881 to 1903, exploring and planting.

In 1903 he went to Northern Nigeria as a Forestry Officer. Although his duties took him all over the country and exposed him to much hardship, he enjoyed excellent health during his first tour of twenty months. After furlough in England he returned to Northern Nigeria in October, 1905, and kept perfectly well till 24th September, 1906. About the latter date he got an irregular fever which resisted quinine in full doses. Not improving he was invalided, and arrived in England on 29th November, 1906. He had slight fever on the voyage home, and at that time observed certain red patches on his skin, which he attributed to pressure from his clothes. After six weeks in the country, during which he took long walks and, with the exception of a slight fever at Christmas time, felt very well, he was sent to me officially for report.

I saw him first on January 15th, 1907. On examination I found his trunk covered with rings and patches of erythema; several cervical, axillary and inguinal glands were enlarged though painless; spleen and liver were also slightly enlarged and his pulse was quick—100. I found trypanosomes in his blood. There were no other symptoms of trypanosomiasis or of other disease. He said he felt fairly well. I sent him to hospital for treatment and observation.

Atoxyl injections were begun on January 25th, 1907. They were given every second day, the dose being gradually raised to seven grains. He remained in hospital from January 21st till March 4th, 1907. For the first fortnight temperature was somewhat erratic, ranging from 98° to 99° F.; once it was 100·8°. Subsequently, with the exception of one short rise of a few hours to 101·8° on March 5th, and attributed to a cold, temperature was steadily normal or subnormal. Erythema and trypanosomes disappeared about February 12th.

After leaving hospital he returned to his home in the country, where the injections were continued. About July symptoms of peripheral neuritis—not of a very pronounced character—showed themselves. He was again taken into hospital and a course of mercury substituted for the atoxyl. The gums were slightly 'touched.' The neuritis subsiding, the atoxyl was resumed in smaller doses. He now became so well that he wished to return to

Africa. The atoxyl was stopped for a fortnight with a view to testing his supposed cure by injection of his blood into animals. At the end of the fortnight trypanosomes were again found in the blood and the atoxyl resumed. I saw this patient lately (16th January, 1908). He says he never felt better in his life; he looks quite well, and no indication of trypanosomiasis can be detected on careful examination.

X.—A. P. P., an engineer, aged 35, arrived at Boma, Congo River, on June 22nd, 1907. He was then in perfect health, and proceeded at once up country to a point about 150 miles above Stanley Pool, where he and his companions camped on the river bank from June 28th till July 17th. Four days after his arrival there he began to ail with anorexia, depression, languor, drowsiness, and on 12th July took to bed with fever (102°). On July 17th his temperature had reached 107°. Next day he was brought to Leopoldville (104°), and on the following day—19th July—Dr. Broden found trypanosomes in his blood. He received a large dose (I understood 1·5 gramme) of atoxyl hypodermically on the 20th. This gave rise to violent gastralgia, but on the 21st temperature had become normal and has remained so ever since, with the exception of slight brief rises (99·5 to 102·4°) which recur with some regularity every fortnight or three weeks. On 23rd July daily injections of ·25 gramme of atoxyl were commenced and, with occasional intermissions of a day or two and increases of the dose to ·5 gramme, were continued till his arrival in Liverpool about the middle of October.

Trypanosomes were unusually persistent in the peripheral blood in this case. Dr. Broden found them when the patient was at Leopoldville every time he looked for them. Dr. Breinl, who examined him on his arrival in Liverpool, found them, notwithstanding intensive doses of atoxyl, on each of five successive days; and I found them whenever I examined the blood—that is, on every alternate day—between October 23rd and November 8th.

When I saw the patient for the first time on October 23rd he informed me that he had had no cutaneous eruption, and that only once—during the initial fever—when one slightly enlarged gland was detected on the right side of the neck—any adenitis. An inflamed patch of skin on the dorsum of the right foot, which began on the 20th of September, and which he attributed to prickly heat, had

almost disappeared when I saw him. The spleen was slightly enlarged, and he looked more anaemic than the blood count (4,000,000) indicated. Appetite was poor, and he felt weak and depressed. There was no palpitation or breathlessness, and no headache even when he had fever. The only pain he remembered was intense aching in the legs coming on every night and keeping him from sleeping; this disappeared when he left Leopoldville and has not recurred.

On November the 8th, trypanosomes being present in small numbers in his blood and temperature being normal, for the first time during his illness the characteristic erythema showed itself on the skin of the trunk. At the same time and for a day or two previously he had been profoundly melancholic, so much so that I feared the nervous system was becoming implicated and that the terminal phase of the infection was about to set in. He was so depressed I was afraid to allow him to go out alone.

On the day the erythema appeared (8th November) I gave him a hypodermic injection of half a grain of sodio-tartrate of antimony, and also on the following days 1, 1½, 2, and 2 grains respectively, and again after two days another 2 grains. The apparent effect of these injections was remarkable. By the third day the erythema had disappeared, his spirits had become good, and for the first time trypanosomes could not be found in the blood. This hopeful condition persisted till November 26th, when depression, though not so intense as on the former occasion, returned and trypanosomes in greater number than I recollect to have seen them in human blood were once more found. On November 26th and 27th he had two grains of antimony by the mouth. I was afraid to resume the antimony hypodermics on account of the intense irritation and pain they gave rise to. Given by the mouth the drug caused nausea and seemed to increase the depression. It was stopped, therefore, and atoxyl resumed. By the 30th trypanosomes had again disappeared and the patient was feeling much better. He left for New York on December 12th, with instructions to continue the atoxyl. A letter just received states that he is feeling much better.

The more important facts (especially as bearing on treatment) of these ten cases are summarised in the following table:—

Case.	Where acquired.	Duration.	Treatment.	Present state.
1	Congo	7 years	Arsenic (*Liquor arsenicalis*)	Well
2	Gambia	1 year 8 months	Arsenic, quinine, urotropine	Dead
3	Congo	2 years 3 months	Arsenic, quinine, methylene blue, etc.	Dead
4	Uganda	3 years	Arsenic, trypanroth, atoxyl	Apparently well
5	Uganda	4 years	Trypanroth-arsenic	Dead
6	Congo	2 years 6 months	Atoxyl	Apparently well
7	Benguela	2 years 3 months	Atoxyl	Apparently well
8	Lualaba River (Congo)	1 year 3 months	Atoxyl, parafuchsin, perchloride of mercury, antimony	Apparently well
9	Northern Nigeria	1 year 4 months	Atoxyl, perchloride of mercury	Apparently well
10	Congo	6 months	Atoxyl, antimony	Improved

Any conclusions we may be tempted to draw from these and other recorded cases as to the effect of treatment in human trypanosomiasis must be tempered by the consideration that we do not as yet know the limit of the duration of the infection in man, that it certainly may run a course of three or four years, and that the lower animals, especially the smaller laboratory kinds, as regards their reaction to drugs, form no very reliable guide to the action of the same drugs in man.

Subject to these considerations we may provisionally infer that:

1. Trypanosomiasis in man is not necessarily a fatal disease.

2. Atoxyl has a marked effect in checking the clinical manifestations of the infection and in causing the parasites to disappear from the peripheral circulation.

3. Notwithstanding continuation of atoxyl treatment, parasites may reappear again and again at uncertain intervals, and usually concurrently with a rise in temperature.

4. Nevertheless, if the drug be persevered with, the parasites ultimately disappear for good and do not return.

5. Large doses of atoxyl are not necessary to secure this result.

6. Large doses of atoxyl should be avoided, as they are apt to cause serious lesions, peripheral neuritis, optic atrophy, gastro-intestinal inflammation, and other toxic conditions which necessitate suspension of a valuable remedy.

7. Trypanroth, mercury and parafuchsin seem ineffective in human trypanosomiasis.

8. Antimony may have a therapeutic influence in trypanosomiasis, but the hypodermic injection of the sodio-tartrate is impracticable.

The prospects of atoxyl treatment I consider most hopeful. As regards efficiency, promptness and mode of action, it seems to me that it is almost on a par with mercury in syphilis and quinine in malaria; and I think in using atoxyl we should conform our practice to what experience has taught us to be the best methods of using these other efficient and long tried remedies.

I do not believe we can kill the trypanosome outright by one or two large doses of atoxyl, any more than we can kill the treponema of syphilis or the parasites of malaria by large doses of their respective specifics. Mercury does not immediately cure syphilis, nor does quinine immediately cure malaria; but they deprive the respective parasites of their pathogenic qualities and keep the patient alive and in good health till, in process of time, the parasites either die out or become permanently inert. So I read the action of atoxyl in trypanosomiasis, and so I would regulate its administration, being careful, as we would with mercury or quinine, not to push the drug too far, and thereby necessitate its suspension. Case 7 distinctly shows that a dose of 2·3 grains given twice a week controlled the disease; why then risk poisoning by a larger dose? Some of my patients have been for months on 2 to 4 grain doses two or three times a week and have done well.

I would therefore suggest for the routine treatment of trypanosomiasis, a two to three grain dose of atoxyl every second or third day and kept up for at least two years. At the same time concurrent specific disease such as malaria, syphilis, &c., should be carefully treated; and, further, the patient should be brought home to his native country, be spared fatigue, worry, exposure, excesses of all

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kinds and be placed in the most favourable hygienic conditions possible.

I fear to overstate my opinion of the value of atoxyl given in this way in the treatment of trypanosomiasis. I am fully conscious that the evidence in its favour, though strong, is far from complete. However, besides direct evidence derived from its actual use in the disease itself, there is a good deal of collateral and indirect evidence derivable from its employment in other protozoal diseases—syphilis for example. Recently I had an experience of this kind which has greatly impressed me. The case was one of Kala-azar—admittedly an almost invariably fatal disease. The patient had been ill for many months. He had the usual hectic type of fever, was miserably emaciated, and had enormously enlarged spleen and liver; the spleen extended beyond the umbilicus. Liver puncture yielded the Leishman body in profusion, so that there could be no question as to diagnosis. He was given atoxyl injections over a long period, at first apparently with little benefit. Severe inflammation of gums, cheeks and palate, together with symptoms of peripheral neuritis setting in, the injections were stopped. Shortly afterwards the patient left hospital, in my opinion then, apparently to die. Soon after leaving hospital symptoms began to subside; he lost fever and sweats; he gained strength and appetite; his spleen and liver shrank, and, when I saw him a week ago, he had been free from fever for five months and appeared to be quite well, although still a little weak. The liver was almost normal in size, and the spleen could just be felt under the ribs. Was this atoxyl? If so, there is hope now for the victims of yet another formerly hopeless disease.

Before concluding this paper, there are one or two points I would like to draw attention to.

It is not a little remarkable that of the ten cases of trypanosomiasis in Europeans which have come under my personal observation three of them were females. Considering the very small number of European females and relatively large number of European males in tropical Africa, this large number of females attacked with trypanosomiasis is a striking circumstance. I am dealing, it is true, with very small numbers, and it is quite possible that the relative disproportion I remark on is accidental; but when we reflect that whereas women in Africa expose themselves, as

compared to men, comparatively little to the conditions favouring the attack of *Glossina palpalis*, the disproportion becomes still more striking.

Another point that has attracted my attention in connection with these cases is the frequency (four in the eight cases in which the point was inquired into) with which the symptoms were immediately ante-dated by what was described as a bite on the leg. The biting animal may have been a Glossina, but in the case of females—and two of the bitten ones were females—one would suppose that the petticoat would afford a protection even more effective than the trouser does in men.

Too much weight must not be attached to what may have been mere coincidence; but these facts are curious, and suggest further inquiry as to the possibility of some blood sucker, perhaps some species of house vermin, being an occasional vector of *Trypanosoma gambiense*.

REPORTS OF THE 'SLEEPING SICKNESS' EXPEDITION TO THE ZAMBESI FOR THE YEARS 1907-1908

BY
ALLAN KINGHORN, M.B., TORONTO,
JOHNSTON COLONIAL FELLOW, UNIVERSITY OF LIVERPOOL
AND
R. EUSTACE MONTGOMERY, M.R.C.V.S.

OBJECTS OF THE EXPEDITION

The objects of the Expedition were to determine to what extent sleeping sickness had invaded British South African Territory, to map out the distribution of tsetse flies and to advise on means for checking the spread of sleeping sickness.

The Expedition was put under the charge of Dr. Kinghorn and Mr. Montgomery—the latter paying special attention to Trypanosomiasis in animals.

The first letter from the Expedition is dated November 22, 1907, from N'dola, North-Western Rhodesia, and is from Mr. Montgomery, relating to a correspondence between the members of the Expedition and Mr. Moffat, the local representative of the various Copper Properties in North-West Rhodesia and the General Superintendent of the Bechuanaland Coaling Co.

Mr. Moffat, who has taken a very keen interest in the sleeping sickness question, has helped the members of the Expedition in every way.

In a letter from Broken Hill, Rhodesia, dated October 23, 1907, and directed to Mr. Montgomery, Mr. Moffat states the following in regard to the question of the tsetse fly and big game:—'I am especially keen on the question as to the connection between the game and the fly, though I am confident that there is a close connection, and that the fly will not exist long without the game, there are undoubtedly here and there spots where there is little or no game where the fly still remains. This possibly is only temporary; the fly will probably disappear from such places shortly.' Mr. Moffat then proceeds to discuss the question of establishing an Observation Camp at Broken Hill, asking for particulars of the probable cost of maintenance. In a letter dated November 21, 1907, from Broken Hill, Mr. Montgomery replied:—

'Broken Hill, Rhodesia,
'*21st November*, 1907.

'Dear Mr. Moffat,

'Your letter of 23rd ultimo reached me at N'dola. I am extremely glad that you realise the imperative needs of research into the "fly" question, for, as you will see, the success of this territory, commercial and industrial, will be extensively modified should good come of any preventative or curative measures.

'I attach herewith a short draft of what I should consider the more salient points in the projected investigation and an estimate of the probable expense. But I would ask you to clearly understand that this estimate might, after a period of work, prove quite erroneous, for, as has been noted in this district already, there are several forms of "fly" disease, and the greater the number of varieties discovered the larger the expense of experimental animals, as each would demand a separate series in the treatment and morphological researches.

'With regard to your kind suggestion that I should undertake this work should it ever receive sanction, I may say that it would be a real pleasure if it may be assumed that the question be approached in an earnest and scientific spirit, and that its inception would imply a free hand to do what was considered necessary to obtain results which would be, not only of scientific and indirect, but also of practical and direct value; but I do not wish to associate myself with any programme which would demand the waste of several months and not include in its object scientific research.

'I am positive that for such an undertaking the Liverpool School of Tropical Medicine would lend their whole support, and though I do not anticipate any movement on your side until the completion of the tour upon which I am at present engaged on their behalf, yet should arrangements be made more rapidly no difficulty would arise, for the results to be striven for are in both cases identical.

'Yours truly,
'R. C. MONTGOMERY.'

'An investigation into "fly" disease, having for its object a curative or preventative remedy, would embrace the following main points, each of which could be approached more or less independently, whilst the results of each would overlap and serve as controls to those arrived at from other sources:—

'1. The nature of the various varieties of "fly" disease parasites met with in domestic or wild animals in the territory; their effects upon domestic stock; and the action of certain selected drugs upon them, more particularly the employment of atoxyl and mercury and the use of the more easily obtained aniline dyes.

'2. The incidence of trypanosome infection in wild game; the nature and effect upon stock of the parasites found.

'3. An enquiry into the transmission of fly disease by means of biting flies, more particularly Tsetse, "Hippo" and "Blind" flies, and the "Stinging" house fly.*

'In the event of success attending the experiments at least one year would be required for thoroughly testing and proving the method of treatment; and since these would follow on those already conducted near Broken Hill, we commence from a working base which eliminates a considerable number of tentative experiments.

'The other main points would receive attention concurrently.

'It is not to be expected that this period of time is sufficient to do more than elucidate a few of the many questions associated with biting flies and big game. One could only work from hand to mouth, and from deductions drawn, institute control experiments which might answer the questions—can flies other than tsetse originate an outbreak of disease? and can flies other than tsetse continue the transmission in a herd when once originated?

'It is known that game harbour the parasite: we could only ascertain figures showing the proportion of each variety affected and the effects of these germs on domestic stock. To ascertain these with accuracy a very large number of head would require to be examined; but at any period of the investigation figures having certain values would be available.

'I would then suggest one clear year as a minimum and estimate accordingly, leaving it to be understood that in research work it is impossible to precisely specify periods of time.

'The requirements of such an investigation would be:—

'A main camp situated clear of all danger from contamination by wandering tsetse flies, in the vicinity of a plentiful water supply and grazing for experimental animals, preferably within easy reach of

* Hippo = *Tabanus*; Blind = *Haematapota*; Stinging housefly = *Stomoxys*.

transport facilities and allowing of suitable accommodation for all engaged.

'Could such a site be found, it would be desirable to place the main camp at a spot from which tsetse fly could be obtained in sufficient numbers to permit of transmission experiments being conducted there without the necessity of erecting a second station within a fly zone and rendering that fly proof; but for treatment experiments it is a *sine quâ non* that no possible chance of re-infection, natural or accidental, should occur.

'The accommodation required would consist of (1) a laboratory building with doors and windows fitted, (2) an office room, (3) huts for the man in charge, (4) sheds for the use of both healthy and infected animals (certain of them to be rendered fly proof by means of wire mesh), (5) accommodation for all servants, (6) stores, buildings for small animals, netted runs for the same, &c., &c.

'All these could be made of timber and daub by native labour, and should not cost more than £150, excluding the imported doors and windows, the necessary gauze and wire netting, and possibly, sundry sheets of iron roofing in the event of thatch proving unsatisfactory, over either office or laboratory during the rainy season. The fittings of these buildings could be readily made from rough sawn timber, but should include at least one good case for books, papers and sensitive instruments.

'The laboratory equipment would be simple, and for ordinary purposes need not demand an initial expenditure of more than £100. Should it be found necessary to go more intimately into the morphology of either parasite or fly this amount might be doubled.

'The number of experimental animals would, of necessity, be uncertain; but, at the present moment, I consider that 50 head of cattle, 15 head of sheep and the same number of goats, 6 horses or donkeys, and several dozen dogs, monkeys, rabbits, guinea-pigs and rats would be sufficient. These would have to be obtained from a source precluding any chance of natural infection, and all precautions would have to be taken en route: factors which would slightly raise the cost.

'A vote of £500 would probably cover the purchases of all experimental animals.

'In addition, the sum of £50 should be added for the cost of

incidentals and sundries in the shape of buckets, gear, ropes, stationery, petty furnishing, &c.

'For the control and the maintenance of the animals and the upkeep of the station, a capitao, 2 cattle boys and say 20 ordinary men would be needed involving a monthly expenditure of about £20.

'This indicates that the sum of £1,000 is necessary, of which 50 per cent. would be sacrificed in animals, and 25 per cent. for current expenses, but it must most clearly be understood that for the thorough investigation of such a wide field as the present subject embraces, as generous a treatment as is consistent with common sense should be given, and that a feeling of parsimony would tend to curtail necessary experimentation and seriously impair the obtaining of positive evidence which must be founded on proof derived from observation and experiments. It may be pointed out also that should no satisfactory results be obtained within the specified twelve months, only current expenses would continue until the estimated number of animals is exhausted, a circumstance which should not occur until the present views on the treatment of fly disease have been subjected to thorough test.

'For ascertaining the incidence of the fly parasite in wild game, I would suggest the establishment of sub-stations, or temporary camps, in say six districts of this territory; two at least of which should be in localities absolutely free from tsetse fly. At times convenient to the investigator, trips would be arranged to these localities and as many head of game as possible belonging to different species subjected to examination and inoculation into small animals which would be brought to the main camp for thorough observation.

'Should the main camp not be situated conveniently in regard to a plentiful supply of tsetse, a second, but more temporary, set of buildings would be required in a suitable locality. This would comprise fly-proof sheds in which to maintain both experimental animals and fly, and would involve the purchase of additional quantities of small mesh netting.

'To conclude, I would consider that a grant of £1,000 or £1,200 would enable the main points known concerning fly disease in other parts of the world to be controlled here, and to test thoroughly the present suggested methods of treatment. Should it be necessary to enter the field of research more deeply this sum might prove

inadequate, and in any case, as already remarked, the investigator should be given as free a hand as possible and receive full confidence in the undertaking, and in no case should he be restricted in the event of the unforeseen occurring. To this sum would have to be added the expenses of the investigator and at least one intelligent assistant who, of necessity, need possess no qualifications.'

In the following Memorandum Mr. Montgomery furnishes Mr. Moffat with a description of the extent and nature of Trypanosomes in cattle in the Broken Hill district.

'(1) Trypanosomiasis of cattle (fly disease) is very prevalent in the vicinity of Broken Hill at the present time. On all sides information relating to losses caused is accumulating, and the disease has been verified in 36 cases.

'(2) It is possible to isolate two distinct species of Trypanosoma from these cases: the clinical effects produced upon naturally affected cattle being similar, but the reaction in inoculated animals, particularly goats and sheep, differ. Both forms are distinct from the classical cases of *T. brucei* infection found in fly disease in Zululand. It is therefore possible that further research would show a greater number of trypanosomes pathogenetic to domestic stock and that the discrepancies noted and described by hunters and travellers when referring to the effects of the tsetse fly are due to this reason.

'(3) One of these trypanosomes, closely allied to *T. dimorphon* found in Senegambia, is fatal to cattle, sheep, goats, dogs, rabbits, guinea-pigs, and rats. Two donkeys inoculated have shown no reaction. The other, possibly identical with *T. vivax* found in the Kameroons, was fatal to cattle in five of the six cases in which it was observed; the other is still living. Sheep and goats have taken the disease; but in eight experiments six appear to have recovered, two only dying (in each instance small weakly animals, which also harboured intestinal worms). Dogs, rabbits, guinea-pigs, and rats appear refractory. One donkey inoculated has shown no reaction.

'(4) Experimental treatment was adopted in most of the naturally affected cattle, and it was eventually shown that atoxyl administered in 20 per cent. solution intravenously, in doses of two three and four grammes on successive days, caused the complete disappearance of trypanosomes from the blood; they however

recurred in from five to ten days, and further doses of atoxyl caused signs of poisoning.

'According to the observations of the Liverpool workers, it is surmised that mercury in the form of corrosive sublimate will prevent the recurrence, when once the trypanosomes have disappeared from the general blood stream. It has been shown that cattle will tolerate the administration of one gramme of this salt given by the mouth, in 1 in 500 solution, on three successive days. It was necessary for me to leave Broken Hill before the combination of these two drugs could be tried in these doses, but the course of the experiments indicate that the greater the amount of mercury given, the longer the period of freedom from trypanosomes in the blood, and it may be shown that even more mercury than this could be tolerated by cattle, and would be necessary to bring about absolute absence of the germs and complete cure.

'(5) No exact experiments with regard to the mode of transmission of this disease were made. Two healthy cattle were walked through a narrow fly belt, and three tsetse flies (*Glossina morsitans*) were observed to bite. Fifteen days later both showed *T. vivax* in their blood and on the eighteenth day *T. dimorphon*. This is about the normal incubative period, and I have little doubt but that they derived the double infection at the time when the tsetse flies were seen to feed.

'The investigations made into one outbreak, and all circumstances relating thereto, throw a very strong suspicion upon two species of biting flies as transmitters of this disease. These species belong to the genera *Stomoxys* (stinging house-fly) and *Lyperosia*, a very diminutive fly, similar in shape to the ordinary house fly. These two genera frequent farmyards, stables, and habitations, and breed in manure pits and dung. The former is incriminated in the natural spread of "fly disease" in Central South America and the Philippine Islands.

'Another genus of biting fly which has become excessively prevalent as I leave here is the *Tabanus*, or "gad fly." This fly is responsible for spreading "fly disease" in Algeria and India.

'(6) My brief work and observations here have shown how urgent is the need for more research into this disease which is impeding the development of the country around rail head, and how imperative it

is to continue experimental work on treatment, which shows with this combination of atoxyl and mercury indications of success which have never been obtained by any previous method of treatment.

'It must be understood that these notes are merely for guidance, as it is impossible to make more dogmatic statements after so short a period of research, and that any deductions drawn therefrom are susceptible of modification.'

It will be gathered from the preceding statement that the loss from disease is increasing—that they had verified its existence in 36 cases; that they had isolated two distinct species of trypanosomes —one closely allied to *T. dimorphon*, the other possibly identical with *T. vivax*; that large doses of atoxyl were beneficial; that with regard to the question of transmission, they walked healthy cattle through a narrow fly belt, and that three tsetse (*Gl. morsitans*) were observed to bite. Fifteen days later *T. vivax* was found in their blood, and on the eighteenth day *T. dimorphon*; further, after investigating an outbreak, they concluded that there was strong suspicion that two genera of biting flies acted as transmitters—one *Stomoxys*, the other *Lyperosia*. They also conclude that *Tabanus* may take a part in transmission, as it does in Algeria and India.

The second communication is from Dr. Kinghorn and is dated December 18, 1907, at Madona, and relates to the rôle of *Gl. palpalis* and *Gl. morsitans* respectively, in the transmission of sleeping sickness, and to certain practical measures for preventing the spread of sleeping sickness.

'Madona, *December* 18, 1907.

'I left Madona on the 20th of November and went down the Luapula as far as Kazembi's, about two days south of Lake Mweru. From there I went East as far as Mshota's and then returned to this place along the Luengo river.

'I did not look for *Gl. palpalis* along the Luapula particularly, as that had already been done, and I understand from Dr. Spillane that this fly exists along a large part of its course. However, I caught specimens of *Gl. palpalis* on the bridge over the Luongo and again opposite the Johnston Falls, both places on the main road from Madona to Kalungwisi. I also got specimens of *Gl. morsitans* in a village just at the commencement of the Falls

After leaving Old Fort Rosebery, I did not see any fly along the whole of the rest of the route.

'I found two cases of trypanosomiasis on the road to Kazembi's. Both these had decidedly enlarged glands and trypanosomes were present in the gland juice. Both men had worked at Kambove. The finding of these cases is a verification of my earlier opinion that cases would be found scattered over the country among the natives who had worked in the Congo Free State.

'We have then, along the Luapula, all the factors which are necessary for an extension of the disease, i.e., cases of human trypanosomiasis and tsetse flies. *Gl. palpalis* is found chiefly along the borders of the river, but also extends for a variable distance up some of the larger affluents. *Gl. morsitans* is found more or less plentifully over most of the country immediately behind the Luapula.

'There is an impression here that *Gl. palpalis* is the only species of tsetse fly which is capable of transmitting human trypanosomiasis. As you are aware, this question has not been settled decisively; on the contrary, from the results of work done in Uganda it seems clear that species other than *Gl. palpalis* can carry the disease and also that mechanical transmission is possible. While I do not believe that mechanical transmission alone is sufficient to account for the spread of the disease, it must not be forgotten that it is a possible means, and in the light of this I am afraid I must criticise the action which is being taken here.

'The Tanganyika Concessions, Limited, have here some six to eight thousand loads which have to be carried to Kambove. Permission has been given to recruit the labour for this in British territory. A strip along the Luapula, some ten miles in width, has been declared to be infected territory. If the natives required to carry these loads to Kambove are recruited in this "infected strip," they will leave here to proceed into uninfected territory on the Congo side of the river If they are recruited outside the infected area, they will have to come into this "infected strip" for the loads. In either case natives will be moving from uninfected to infected country, or *vice versâ*. This, of course, is the one thing which should not be allowed.

'It is said that there are no *Gl. palpalis* on the route which is to be followed from here to Kambove. While this is the case,

Gl. morsitans is very plentiful, and the width of the road which is being cut (12 feet) is not by any means sufficient to banish them. I should strongly advise that the Tanganyika Concessions, Limited, be required to move the loads across the river and to find the carriers in the Congo Free State. I was informed by Mr. MacDonald, the agent of the Tanganyika Concessions, Limited, that food is very plentiful on the Belgian side of the river, and consequently natives also, so that there should be no difficulty in getting the carriers. However, this is a matter which does not intimately concern the Administration here, except in so far as a certain amount of money would be diverted from the country. It must be clearly understood, though, that if the disease is to be prevented from getting a foothold, all commercial considerations must give way until such time as the etiology and treatment of human trypanosomiasis are on a more satisfactory basis.

'The other proposal that I feel is unjustified is one to establish a temporary segregation camp near Madona for the cases now known to exist. It is impossible to have this camp more than four or five miles distant from this station, as otherwise it would be too far away for easy access, and since natives are returning from Kambove, a medical officer is required here to examine them. The only place available within this distance, with a suitable water supply, is on the Luafumu river. Just around here, however, *Gl. morsitans* is fairly plentiful, so that there exists a risk of the disease spreading, however minimised by extensive clearing. To digress for a moment, I have a specimen of *Gl. palpalis* caught on the verandah of the assistant magistrate here, although the whole front of the river has been cleared for some distance up and down from his house (the house lies 200 yards from the river bank, which has been absolutely cleared for 400 yards in all, and beyond this cleared for gardens). Mr. Hughes, the assistant magistrate, informs me that there are places in the Luapula division eminently suited for a segregation camp, i.e., there are no tsetse flies, no villages and plenty of water. Of course until more medical men are available, some such course (i.e. a temporary segregation camp) will have to be adopted if the cases are to be treated at all, but the British South Africa Company should take steps to increase its medical staff immediately, so that the situation may be met energetically.

'In order to avoid misunderstandings, it should be clearly defined that the matter is one for which the medical officer is responsible and not the native commissioner. There is some tendency to reverse this order of things, but this should not be. The situation should be entirely under the control of the medical officer, and any assistance granted him from the native department should be subject to his direction.

'I was informed by Mr. Beringer, whom I met at Kazembi's, that the natives are crossing the northern frontier very freely. There are a lot of Swahili traders there engaged in smuggling, and these encourage native movement. An ordinance has been passed which will require these to leave the country. This of course is in accordance with the advice given by Todd some time ago, and was a very necessary proceeding. If it has not been done, I think some legislation should be passed which will enable the officials in charge of the sleeping sickness work to deal with any European who may feel disposed to ignore the regulations which have been made regarding the crossing of the borders with natives. So far as is known here, there is no penalty attached to this, so that if a European should go, say from Madona to Broken Hill through the Congo Free State, there is no means of dealing with him. In several cases special permission has been given for this to be done, and as the natives were recruited in the infected area, a dangerous precedent has been created. The Administration of North-Western Rhodesia should consider the advisability of forbidding the entrance of any native from this infected territory into its country.

'The whole question may be put thus—As a result of the finding of *Gl. palpalis* and cases of human trypanosomiasis along the Luapula river, a strip ten miles wide bordering the stream has been declared infected territory and all movement from it stopped. Yet it is proposed to allow the Tanganyika Concessions, Limited, to move some six thousand odd loads with carriers from North-East Rhodesia through country abounding in tsetse flies to a district from which the cases now present in the country have come. In addition natives have been allowed to proceed from the infected territory through non-infected parts of the Congo Free State and North-Western Rhodesia. In view of what we know at present of the etiology of sleeping sickness, I think that this is a mistaken policy. I would urge the adoption of the following suggestions:—

'1. *The Luapula should be closed absolutely* until it has been proved definitely that *Gl. morsitans* cannot spread human trypanosomiasis. In my last letter I suggested how the river might be closed.

'2. Legislation should be provided whereby any person taking natives across the border can be prosecuted and fined.

'3. The Tanganyika Concessions, Limited, should be required to find carriers for their loads in the Congo Free State.

'4. The Administration of North-Western Rhodesia should forbid the entrance of any native into its territories from any infected area in North-Eastern Rhodesia.

'5. Segregation camps for patients should be stationed in a district free from tsetse flies of any species. In order to do this the British South Africa Company will have to increase its medical staff.

'6. The medical officer should be given charge of the work and afforded such assistance from the native department as may be necessary to carry out his instructions.

'As I have said before, I do not think that the disease has any hold on the country as yet, and if the matter is faced at once regardless of any other considerations, it can be prevented from spreading to any extent. Otherwise the danger of this is great, so far as our present knowledge extends.

'Yours faithfully,

'(Signed) ALLAN KINGHORN.'

In a letter dated Madona, January 28, 1908, to Dr. Barratt, of the Expedition which was sent out to investigate Blackwater Fever in Nyassaland, and if practicable to act in concert with the Sleeping Sickness Expedition, Dr. Kinghorn makes a statement in which he emphasises the importance of regarding all biting flies with suspicion, and states that every effort should be made to find exactly what flies, tsetse or others, are capable of carrying the virus.

'As regards "fly," *Gl. palpalis* has been found on both sides of the Luapula from Kapwepi's to Kasiwa's, a village about two days south of Lake Mweru (by Dr. Spillane and myself). It has also been found by Dr. Spillane along the whole of the British coast of Mweru, around the south end of Lake Tanganyika, and also along parts of the larger affluents of the Luapula and Kweru, notably the Mansa and the Kalungwisi. I found it on the Luongo a short distance from its mouth. From Kasiwa's to Mweru, on the British side, the Luapula

is bordered by swampy plains some miles in width, so that the conditions are unfavourable for this fly. On the Belgian side, this does not obtain to such an extent, so that the fly is probably present, although I have no definite knowledge as to this. *Gl. morsitans*, and possibly the closely related *pallidipes* and *longipalpis*, is widely spread over the country, however. On the road from Broken Hill to Fort Jameson, I found them at one spot in the Machinga Hills, from the base of these right across the Luanga to within a day of Petauke, and near the Sasare mine. From Fort Jameson to the Luapula, they occur from the Luangwa to the base of the Machingas. There is a sudden rise here to the plateau of 2,500 feet or so, and on top of the hills I did not see fly again until I got to the Kasanka river. In this vicinity they are very plentiful, and stretch over to the Luapula and up north past Chitambo's through the country to the east of Bangweulu. They may be said to be found in most of the Luapula division.

'I have found three cases of sleeping sickness in the course of my work, all with a history of having worked in the Katanga mines. All had markedly enlarged glands, and, at the time I saw them, appeared perfectly healthy. Fly (*Gl. morsitans*) was present in the village of at least one case. Considering the thousands from this country who have worked in the Katanga mines, I am perfectly convinced that there are many more cases scattered through North-East Rhodesia, as the labour was not drawn from any one particular district.

'As you are aware, all the work which has been done goes to show that the transmission is mechanical. While I think this is not sufficient to account for the rapid spread of the disease in view of the great difficulty experienced in getting positive results, it is a fact which cannot be too strongly emphasised. There is too great a tendency to regard *Gl. palpalis* as the only infecting agent. That this is not so has been shown by the work in Uganda, where successful transmission experiments were made with *Gl. fusca*. Again the work on cattle trypanosomes shows that while one or two species are normally concerned in the extension of the disease, it is quite possible to effect this by the use of not only other species of tsetses but also other distinct genera, e.g. *Stomoxys* and *Tabanus*. This surely is sufficient to demonstrate that, at present, all biting flies, and

particularly all species of *Glossina*, must be regarded with suspicion. While, perhaps, the task of controlling the spread of sleeping sickness would become impossible if every biting insect had to be considered, it is not so with regard to the tsetse flies, and I would hope that on every occasion on which you have the opportunity you would emphasise the importance of not simply regarding *Gl. palpalis* alone as dangerous. The question of the etiology of the disease is in a more unsatisfactory condition than the treatment, at all events from the prophylactic point of view, and it is most important that some definite effort should be made to find exactly what flies, tsetse and others, are capable of carrying the virus.

'As to enlarged glands, I have found that a fairly large percentage of the natives (roughly 30 to 40 per cent.) have palpable glands which come chiefly under the " + — — " group of Dutton's and Todd's classification, though there are also a number of " + — " glands. On puncture these were found to be uniformly negative. This occurrence of enlarged glands rather complicated the diagnosis, for it means that the statement " every negro with enlarged glands must be considered, until the contrary is shown, to be a case of trypanosomiasis," cannot be accepted for North-East Rhodesia, and, presumably, for Nyassaland as well. Consequently a trained medical officer is the only person who can look for the disease satisfactorily, since puncture is necessary. I have advised that the British South Africa Company should have special medical officers to travel through the country looking for cases and getting them isolated at once. Whether it will be necessary for like measures to be taken in Nyassaland is uncertain as yet.

'The chief danger for the Protectorate lies in the spread of the disease to the north end of Lake Nyassa. Trypanosomiasis is endemic on Tanganyika at Vua in the Free State, a short distance above the Congo-Rhodesia border, and between the people on either side of this there is unrestricted communication. *Gl. palpalis* is found right round the southern end of Tanganyika, so that it is extremely probable that cases of the disease are present in that part of the country. Whether the disease is to be found in German East Africa in this neighbourhood is not known. At the north end of Nyassa *Gl. fusca* has been found, and this, we now know, can carry the disease. If once a case gets over where these flies occur an

epidemic might easily be started. I believe natives of the Protectorate are carrying loads from Karonga to Kasana, and as large numbers of natives from the Awemba district of North-East Rhodesia have worked in the Katanga, cases probably exist amongst them, and the possibility of their infecting negroes from Nyassaland must not be overlooked. It therefore seems to me that the north end of Nyassa is the part which requires most attention from the authorities of the Protectorate. It would also be well to bear in mind that natives of the Protectorate have been working in the Katanga and some are only returning home now. Some of these may be infected and, in fact, I should not be at all surprised to hear at any time that cases of human trypanosomiasis had been found in Nyassaland.

'As soon as we get carriers we intend to travel across to the north end of Nyassa in order to examine the conditions there personally. We should be there in four or five months at the outside.

'Yours sincerely,

'(Signed) ALLAN KINGHORN.'

The members of the Sleeping Sickness Expedition, having seen in the 'Times' of 13/12/07 an article dealing with Game Preservation and its relation to Ngana, make the following statement:—

'THE PRESERVATION OF SOUTH AFRICAN WILD GAME.

'It may not be considered out of place for us to enter into the discussion on the subject which appeared under the above heading in the issue of the "Weekly Times" for December 13, 1907, as the article bears most directly upon the question of tsetse flies and the disease they transmit which we were dispatched by the Liverpool School of Tropical Medicine in the spring of last year to investigate on behalf of the respective Governments of British territory, North of the Zambesi.

'We hold no brief, and are actuated by no party: we merely discuss the problem from the scientific standpoint, and if sentiment sways us towards the prevention of vandalism in the works of man or nature, there is not lacking evidence which completes your correspondent's propositions and shows the danger existing. We do not presume to enter the lists on a footing with such observers as

Sir Alfred Sharpe, F. C. Selous, F. J. Jackson, or R. G. Harger, whose intimate knowledge of their respective countries and long residence there places them in a position to have personally watched the passage of events. We arrive as observers of the question as it stands to-day in the parts of the Continent over which we have travelled.

'The problem may be stated in the following paragraphs which appeared in the article mentioned:—

'"1. . . . That certain species of tsetse fly exist only where wild game exists.

'"2. That certain germs exist in the blood of wild animals, which . . . . when transferred by the tsetse . . . . are the cause of Ngana or tsetse fly disease.

'"3. If therefore the propositions quoted (above) can be proved valid the game must be destroyed; there is no help for it."

'1. We have the widespread and uncontroverted statement that in many districts of South Africa the tsetse flies have disappeared; the reasons advanced being (1) the progress of civilisation, which implies the opening up of the country, the presence of men and guns, and the destruction of some game, the flight or retreat of those remaining: and (2) the epidemic of Rinderpest which swept over the country towards the middle of the last decade, killing off buffalo and antelope, and so removing the only food upon which the tsetse was held to exist.

'North of the Zambesi a precisely parallel state of affairs has obtained. Rinderpest raged, killing cattle in front and leaving the veldt strewn with carcases of game. A railway has been built, mines and mining centres opened up, and farms and mission stations are scattered over the territory, each and all implying the advent of men and guns, the destruction and the driving back of game. But the results have not been the same: wherever there is evidence that tsetse existed in the past, there it may be found to-day. Naturally we do not speak of the centre of a busy mining camp: the retreat of fly from this would be as from a native village; it is a relative and not an absolute disappearance. Further, there is much evidence that the tsetse now holds sway over roads and areas where they were unknown a few years ago.

'We are told that the tsetse of South Africa depend upon game for a living: it is true that science does not yet know for certain that this fly can obtain a living on anything but blood. Many other biting flies can maintain a vegetarian existence for a time at least, and the circumstantial evidence is certainly strong that the genus *Glossina* can do the same. In what other way can be explained the not infrequent occurrence of districts uninhabited by man and in which game, spoor or even the ordinary indications which go to make a "game country" are wanting, yet in which tsetse abound in their thousands, congregating around the traveller and his carriers in numbers impossible to ward off? An estimation of the odds against any one of these flies obtaining a meal of mammalian blood in the course of a year would indeed be work for a statistician. Further, there are districts and large areas wherein game of all local varieties is plentiful and in which no tsetse can be found. What explanation can be offered? Only that, like any other being, the members of the genus *Glossina* have their likes and dislikes, and select for a home a locality suitable according to their tastes. Even amongst the members there is individualism, for one species—*Gl. palpalis*—will not live away from the immediate vicinity of water; and only such water, too, as is open, and is bounded by defined banks which carry a sufficiency of timber or scrub to provide him with the necessary amount of shade. As every traveller in Northern Rhodesia knows, the tsetse fly of that country—*Gl. morsitans*—does not make a point of these conditions; but it does insist on shade, preferably that furnished by the half-grown trees which constitute the virgin bush, the ground between which is covered with grass, smaller trees or scrub. It does not live on an open flat, dambo or vlei, and it is quite patent that the presence of water is secondary in its estimation. From this it is obvious that there is much truth in Mr. Dunbar's suggestion that local variations in the tsetses may account for some of the discrepancies in the accounts given by various writers, for he, in turn, introduces another species of tsetse into the argument. *Gl. fusca*, the large species to which he refers, has been shown to exist at both the North and South ends of Lake Nyassa; its haunts and habits are not well known, but there is some indication that it favours similar country to *Gl. palpalis*, i.e., water and shady banks. As some species of game undoubtedly confine themselves to certain

types of country, and as the tsetse is also shown capable of selective powers, may it not be that the association of fly—used without distinction of species—and particular varieties of game is but a natural inclination of both beings to the same locality, and not a peculiar affinity between the individuals?

'These statements do not answer why the tsetse (believed to have been both *Gl. morsitans* and its relative *Gl. pallidipes*) has disappeared from parts of Africa South of the Zambesi, but they go to show that game destruction by civilisation and rinderpest is not the only factor concerned. Until we are better acquainted with the bionomics of the genus *Glossina*, by observation and experiment conducted on scientific lines, the statement "that certain species of tsetse exist only where wild game exists" must remain controversial, but with a bias to the negative, if, indeed, it must not be negatived.

'2. The second of your correspondent's propositions may be dealt with in a more dogmatic fashion. ". . . . that certain germs (trypanosomes) exist in the blood of wild animals which produce in them little or no ill effect; and that these germs when transferred by the tsetse . . . are the cause of Ngana or tsetse fly disease."

'The trypanosomes of various countries all possess features in common with those of Zululand, which was shown by Bruce to be incapable of living outside an animal body, and to be spread by a biting fly, to the exclusion of all other natural means. They differ only in minor structural points, but especially in their actions upon stock. From all, death is the result in a susceptible animal, but towards some trypanosomes, cattle, towards others, camels, possess a degree of resistance approaching or equalling that enjoyed by wild game towards the Zululand form.

'As the germ cannot live in air, forage or water, it can only be derived from a pre-existing case; a reservoir, which like big game may not suffer from the disease, or an animal already sick. Bruce showed that buffalo, koodoo, wilderbeeste, bush-buck and hyaena may be that reservoir in Natal; Dutton and Todd proved that the bush-buck in the Congo Free State harboured the trypanosome of fly disease there; and in North-Western Rhodesia we have found the germ in bush-buck and Lichtenstein's hartebeest. This germ is not the same as that found by Bruce, but the sickness in cattle is identical. In North-Western Rhodesia, and when properly studied

the same will probably obtain elsewhere, there is more than one species of *Trypanosoma*, and though the effects towards some animals—cattle—are the same, the action on others—goats and sheep—is different. May this, too, not account for many of the discrepancies in travellers' reports? For example, the opinions regarding the susceptibility of the donkey are very diverse; we found that this animal took one of the Rhodesian trypanosomes, whilst a second donkey did not become affected with the other form of the germ which occurs there.

'In many countries, and districts of countries too, where trypanosomiasis is rife, the amount of game is negligible. From whence does the virus, the *causa causans*, then arise? When the conditions are examined it is found that there is always at least one species of domestic animal which is more resistant than another: it may never be sick; or if it does take the disease it may live for two or three years like the camel with the Indian fly disease, and it may even recover. In South America, fly disease is seen chiefly in the horse, and cattle rarely suffer, but they, in common with the camel of India, take the place of the non-existent game, and act as a reservoir which under the required condition can issue supplies of trypanosome to all comers; causing death in those susceptible and converting the others into additional reservoirs.

'But what is the required condition without which the presence of a reservoir is not greatly to be feared? Bruce showed it to be a biting fly. In Africa travellers blamed the tsetse; in India natives incriminate the "horse fly" (*Tabanidae*), known in Rhodesia as the "hippo fly," and a smaller variety locally called "blind fly" (genus *Haematopota*); in South America and parts of Asia the "stinging house fly" (genus *Stomoxys*) is blamed.

'With few exceptions no one in South or Central Africa has even suggested the possibility of any fly other than tsetse causing the disease; and since the time when Bruce proved the connection scientifically, what was at first a mere suspicion influenced by native tales has become a conviction, to the exclusion of all other flies. It is not necessary to look far for the reason. Stock owners knew they could keep their animals around the farmstead and in certain areas with impunity, even though horse flies and the stinging house flies were present. Compared with the tsetse these other flies are

"domestic"; they are limited in distribution on the veldt; their haunts and habits keep them from the free association with game that tsetse enjoy in the zones of which they and game are common residents; and they have not a fraction of the voracious nature and pertinacity possessed by the *Glossina*. On *primâ facie* grounds they cannot be incriminated to the same extent as those which live among the reservoir and which will follow an ox for miles in the hope of obtaining a meal. It is this very "domestic" nature of the other flies, however, which renders them accessories par excellence in spreading the disease when a domestic animal, controllable and housed, is the reservoir. Let the farmer in a tsetse fly country expose one of his animals until it acquires the disease and then herd it with the rest in a kraal inhabited by *Stomoxys* or other biting flies such as *Lyperosia* or *Haematobia*. The reservoir and the biting fly are present in the midst of healthy and susceptible stock; and the results have been severe enough to be well appreciated by some agriculturists in Northern Rhodesia.

'3. This second proposition is proved valid: Buffalo, Koodoo, Wildebeeste, Bush-buck, Hartebeest and Hyaena have been shown "accessories before the fact," and if we adopt your correspondent's view, they must be destroyed.

'We enter a plea that sentence may not yet be passed. To quote from the article:—"A hasty sentence of wholesale death is one whose execution, once accomplished, can never be annulled." Every month is adding to our knowledge concerning the disease in all parts of the world, and the rapid accumulation of evidence all goes to show that efficient sanitary police measures adapted to the requirements of each district, and even to each individual owner, formulated and carried into effect by an adequate staff of veterinary officers will do much to control trypanosomiasis in the domestic animals. There is no proof that the eradication of the game will be followed by the disappearance of fly disease: South Africa cannot be accepted in evidence, for the factors said to have been causative there have been without effect in other parts of the Continent. We have indicated that the destruction of the tsetse would not prevent the transmission of the germ under the conditions which obtain in countries where both game and *Glossina* are non-existent; and though it is patent that the extermination of the reservoir would, *à priori*, check the

disease, we have no proof that in Central Africa a storehouse for the germ other than game may not exist.

'Irrevocable measures are not called for to satisfy the materialistic and often speculative desires of the few: for the many, a proper knowledge of the disease and the adoption of trained advice as to how it can often be avoided, may tide over the time till an effectual cure or a specific means of prevention be made known; and we earnestly urge that less draconic measures may be given a full and fair trial before the death warrant of the greatest natural attraction of Central Africa be signed.

R. EUSTACE MONTGOMERY.
ALLAN KINGHORN.

Madona, North-Eastern Rhodesia, *January 28th*, 1908.

In correspondence dated Madona, February 7th, 1908, the investigators state:—

'With regard to the report we should like to remark that we disagree with the policy of the Tanganyika Concessions, Limited, in sending loads to Kambove with natives from this country. By refusing permission to recruit labour for the mines, and still allowing loads to be taken in, we think that the Administration is placing itself in rather a false position. The Tanganyika Concessions, Limited, if required, would, we think, be able to find carriers in the Katanga as they have to do with regard to loads coming in viâ Broken Hill. They say they are taking precautions to prevent these carriers becoming infected, but these precautions only consist in cutting a road about 12 feet wide along a route in which *Gl. morsitans* is very plentiful, and by the establishment of food stations away from the villages. The natural tendency would be for the natives to try and stay in the Katanga to work, and in at least one instance a negro has run away from the gang when returning to Madona. Of course, he may not have gone back to the mines, but this is the likeliest thing, as they are paid on getting back there.

'Another rather anomalous proceeding of the local government is the official declaration of the 10 mile strip along the Luapula to be infected, so that natives come into this area on their way to Kambove, which is "uninfected." Thus we have the passage of negroes from infected to uninfected territory and *vice versâ*.

'Yours faithfully,

'ALLAN KINGHORN.

'Madona, *February 4th*, 1908.

'Dear Dr. Hearsey,

'In a recent number of the "British Medical Journal" I notice a reference to your last year's report, and as you consider the question of sleeping sickness, I should like to get a copy of it, and also of the map showing the distribution of tsetse flies, if you will kindly send them to Abercorn.

'I notice also that the road from Tanganyika to Nyassa is now unused, and that Dr. Todd's prediction as to the infection of the South end of the last-named lake has not been fulfilled. Unfortunately this is not the case. Evidence is at hand that cases now exist there, and as *Gl. palpalis* is found along its shores, the danger of a wide dissemination of the disease is great. It is not surprising that this is true, for although fly was known to exist along the Belgian side of the lake, certainly as far South as Vua, and also that imported cases were present at Moliro (1901) and Baudoinville (1902), nothing was done by the Congo authorities to check the spread of the disease, and only last year the natural consequences of this were shown by a report that the disease had become endemic at Vua. As natives on either side of the border have been communicating uninterruptedly, it would be a matter for some surprise if cases had not been brought into British territory. In German East Africa cases have been present at Ujiji, but whether there is any danger of the disease spreading southwards to the north end of Nyassa I cannot say. *Gl. palpalis* is found on the German portion of Tanganyika, certainly below Bismarckburg.

'Along the Western border here I have found three cases of trypanosomiasis, all with a history of having worked in the Katanga. I consider that these cases originated there without doubt. No indigenous cases have been found here, and as *Gl. palpalis* occurs along the whole of the Luapula from Kapwepwi's to Kasiwa's (two days south of Mweru) and the people are on the river constantly, these would have been expected had the disease been indigenous.

'As a result of the extensive importation of labour into the Katanga, cases of sleeping sickness will probably be found in all the districts from which it was drawn. This applies not only to Rhodesia but to Nyassaland as well. I am not aware how many have gone from Nyassaland, or whether they have been watched since

returning, but whether or not, there is any danger of the disease coming in from the North, this route must not be forgotten. Skilled workmen and raw labour as well, from Nyassaland, are still in the Katanga. The presence in the Protectorate of *Gl. fusca*, known to be capable of transmitting *T. gambiense*, is of importance if cases are found.

'I cannot deprecate too strongly the continued permission to the Tanganyika Concessions to carry loads from Madona to Kambove with Rhodesian natives. The anomaly of this is at once apparent when it is remembered that free recruiting for the mines has been stopped. Another anomaly is that while Kambove is not regarded as being in the "infected" area of the Katanga, a strip 10 miles wide, along this side of the Luapula, has officially been declared to be infected. By allowing natives to pass, therefore, from an infected to a non-infected district the extension of the disease is inevitable. In my opinion the Luapula should be absolutely closed.

'There is a possibility of slight danger being attached to the Karonga-Kasama route. Cases of sleeping sickness will probably be found in the Awemba district of North-East Rhodesia, and tsetse-fly exists along the road in places. At present, however, I am inclined to think the danger is remote.

'When I was in Fort Jameson, Dr. Barclay told me that the results of the palpation of *all* the neck glands, not simply those in the posterior triangle, had been included in the report sent to the Colonial Office early in January (dated Zomba, January 24th, 1907). If this is correct for all the figures, they would need to be corrected. Were any of the " + " cases punctured? In view of what we have found in Rhodesia, this would be advisable. The cases I found had " + " glands, and, of course, showed the parasite (of five " + " cases seen, three were infected). Of 9,005 natives examined by me between July and December, 20·85 % had palpable glands, mostly very few in number and shotty to the touch.

'One other point on which I should like to touch is the question of what tsetse flies are able to transmit the disease. As you will know *Gl. palpalis* and *Gl. fusca* have been shown to be capable of doing so. Whether or no the other species can effect the transmission as well, is not known, but since mechanical transmission is the only method of which we have any knowledge at present, the part these

may play, given the presence of cases, becomes an important consideration. Even though it should eventually be shown that the trypanosomes have a life cycle in any one tsetse fly this mechanical transmission cannot be absolutely ignored.

'Yours faithfully,

'ALLAN KINGHORN.

'The Principal Medical Officer,
'Zomba, Nyassaland.'

THE INCIDENCE AND PROPHYLAXIS OF HUMAN TRYPANOSOMIASIS IN NORTH EASTERN RHODESIA

BY

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(First interim report of the Expedition of the Liverpool School of Tropical Medicine to the Zambesi, 1907-08)

When the Expedition to the Congo returned to England in the autumn of 1905, Dr. Todd stated that there was reason to fear an extension of human trypanosomiasis from the Congo Free State into British territory. This statement was based principally on the facts that the disease was spreading steadily southwards, that imported cases of sleeping sickness existed practically on the borders of Rhodesia, at Moliro and Baudoinville on Lake Tanganyika, and that one of the main trade routes ran from this lake to Nyassa. The history of the spread of the disease in the Congo showed that it had been carried from point to point along the main caravan roads, often with startling rapidity.

In the following year, reports were received that an imported case existed at the Belgian post of Kasenga on the Luapula River, and in September, 1906, Dr. Todd and one of us were informed by Mr. F. W. Arnott, of the Garanganza Mission, that cases were present at Lukafu on the Lufira River. In November, Dr. Todd received from Dr. Massey, then of the Tanganyika Concessions, Limited, a communication stating that the disease was endemic in the villages around Lake Kisale and some of its confluents, and further, that about 9 per cent. of the carriers from Kabinda to Ruwe were infected. (When Dr. Todd went through this part of the Congo, he found that about 13 per cent. of the people in the neighbourhood of Kabinda were infected, but that fish sellers from the South, whom he saw there, were free from the disease.) In about a year and a half then, June

1905 to November 1906, the disease had become endemic around Lake Kisale, having been imported from Kabinda. As loads were constantly being brought to the Katanga mines from these infected areas, the greatest danger existed that foci of the disease would be established there, especially as Dr. Massey had found tsetse flies (*Gl. palpalis* and *Gl. morsitans*) in the immediate vicinity of some of them. Most of the labour for these mines had been recruited in British territory, so that the gravest fears were entertained that the disease would be carried into Rhodesia by some of these men on returning to their homes. Finally, about the same time a report reached home that the disease was invading the Western shores of Lake Mweru.

In consequence of this knowledge, representations were made to the Colonial Office by the Authorities of the School, pointing out the danger of trypanosomiasis being carried into British territory, and asking for support for an expedition which it was proposed to send to the threatened districts to study more fully the local conditions. At the same time the British South Africa Company were asked to assist, as North-Eastern Rhodesia would probably be the first to suffer. This proposition was eventually accepted, and the present expedition sent out. Broken Hill was reached on June 16, 1907, and shortly afterwards one of us left for Fort Jameson.

The following report, it will be understood, does not discuss the situation fully, as we have seen but a small portion of the country. It is believed, however, that enough evidence has been accumulated to judge of the probable occurrence of human trypanosomiasis, and also to judge the value of the various measures which have been suggested for combating the disease.

ROUTE

On the accompanying map the route followed to date will be found, and also those areas of North-Eastern Rhodesia in which species of *Glossina* are known to occur. This information, of course, is not complete at present. So far as possible the main roads were followed, as it is along these that experience has taught us the disease is usually carried. As we went from village to village, all the inhabitants were palpated, and in those cases in which it was possible, gland puncture was performed. This enabled us to control the value

of gland palpation as a means of diagnosis so far as a country was concerned in which the disease was supposed to be non-existent. Most attention has been devoted to the Luapula division, as large numbers of natives from this district have worked in the Katanga, and as, in the natural course of events, it would be the first portion to be invaded. The other area from which the disease was expected to enter Rhodesia was along the border between Mweru and Tanganyika. As yet we have not been able to visit this part, but evidence is accumulating that the prediction made by Dr. Todd is in course of fulfilment.

DISTRIBUTION OF TSETSE FLIES

A. In North-Eastern Rhodesia.

1. *Glossina palpalis.* In brief, we now know that this species exists along a large part of the Luapula and some of its confluents; on the shores of Lake Mweru; and around the southern end of Lake Tanganyika.

It was first found in 1906 by Dr. Noble at Kasenga and the Nafunta Falls on the Congo side of the Luapula. This year it was found by Dr. Spillane along this river from Kapwepwi's to Kasiwa's village, and along the British portion of the two lakes. We have been able to confirm these observations as regards the Luapula. It was also found by Dr. Spillane for some distance up the Mansa and Kalungwisi rivers, and we have found it up the Luongo a short distance from its mouth.

Along the portion of the river mentioned above, the bush extends right to the margin of the water, where it assumes a more luxuriant growth and affords abundant shade. From Chongola's to Sakontwi, the river is bordered by wide flats almost completely destitute of vegetation. From Sakontwi to Kapwepwi's the shore is fringed by a single, more or less continuous, row of bushes and small trees which project obliquely outward over the water. Behind this there is a bare, treeless strip of ground varying in width from 25 to 200 yards, and beyond this again the ordinary thin bush of the country begins. This lack of continuous shade behind the river and the small amount of shelter afforded by the fringe of bushes probably account for the absence of *Gl. palpalis.* From Kasiwa's to Mweru, the conditions are

somewhat similar except that swamps, one to five miles wide, replace the dambos. This fly, *Gl. palpalis*, is most readily found by paddling just along the trees which line the shore, and success often follows this manœuvre when a search on the shore itself has been fruitless. As a rule, the fly will not come out to the canoe if very far from the shore, although occasionally we have noticed it at a distance of from 100 to 150 yards. As has been observed before, we have found it to be most active in the middle portion of the day when the sun is shining brightly. Early in the morning and late in the evening are unfavourable hours to look for it. We have also been able to confirm the observation made in Uganda[1] that the fly is absent from those portions of the shore where the trees are replaced by grass and water weeds for any distance.

2. *Glossina morsitans.* While we use this specific name, we wish it to be understood that the identification of our specimens is not complete, so that in addition to *Gl. morsitans* proper, the closely related *pallidipes* may exist. This will be discussed more fully at a later date.

This fly has an extremely wide distribution, not in definitely defined belts, such as are said to be found farther South, but more or less generally scattered over the whole country. For the purposes of this report it is not necessary to give all the situations in detail: it will suffice to say that it is found in most of the districts in greater or lesser numbers. While these areas are marked on the map, it must not be concluded as yet that they are not present in the unmarked portions.

Like *Gl. palpalis*, we have found them to increase in activity as the sun approaches the zenith, and to be fewer in number and less voracious on dull days. Their activity is maintained well on towards sunset, and we have noticed an occasional one flying into the tent after this time. Water and shade are not such necessary factors to this fly as to *palpalis*. We have observed it at least three miles from water, and have been informed by one of the officials that he has seen it ten miles from water. The amount of shade afforded by the thin type of bush peculiar to the country is very small, in the dry season practically none. A certain amount of shelter is required, however, as this fly is not found on the open dambos which break the continuity of the bush.

The consensus of opinion throughout Rhodesia is that tsetse flies are steadily increasing in numbers, regions which only a few years ago were free being now heavily infested by species of the genus, i.e., with *Glossina morsitans* and possibly *pallidipes*. This increase cannot be ascribed to a corresponding increase in game, since large areas exist where game is practically absent and tsetse flies abound, while in other parts the reverse holds good.

B. In the Nyassaland Protectorate.

Glossina palpalis has not been found here, but other species are known. *Gl. fusca* has been observed in the Karonga district, and again in the neighbourhood of Chiromo. *Gl. pallidipes* was found in the former area, and *Gl. morsitans* is present in various parts of the country.

C. In the Katanga District of the Congo Free State.

1. *Glossina palpalis*. From Dr. Massey we learn that this fly occurs on the West shore of Mweru; at the junction of the Dilukwe and Lufira rivers; at Nkoni Hill on the Lufira; on the Lukulegi river near the Congo-Zambesi watershed; at Busanga, junction of the Lufupa and Lualaba (tin mines); on the South Kaluli, at the cut road crossing from Ruwe to Mazanguli's; on the Lualaba, from the Kalenga Falls to Chisamba; on the Inje river, running into Lake Kisale.

2. *Glossina morsitans*. In the whole region bounded by the Lualaba and Lufira; along the road from Kambove to Madona; in the neighbourhood of Busanga; on the Lubudi river, and at its junction with the Mkuleshi; on a line drawn from Ruwe to Kansanshi, in two places.

The disease is endemic in this part of the Congo along the Lualaba, from its junction with the Lubudi to its point of exit from Lake Kisale, and on the Lufira around the Government post of Kayumba and at its junction with the Dilukwe. Moreover, the whole of the Lufira from Kisale to Mwenda's village, a short distance from the Nkoni Hill Mission and the local administrative post of Lukafu, is suspected. We have also mentioned above that imported cases of the disease were present at Nkoni Hill in September, 1906.

GLAND PALPATION AND PUNCTURE

Since Greig and Gray[2] noted that trypanosomes were fairly constantly present in the enlarged glands of persons suffering from trypanosomiasis, the efficiency of this means of diagnosing the disease in its earlier stages has been repeatedly demonstrated. Dutton and Todd[3] were the first to recognise its practical importance, and their experience led them to make the statement that 'every negro with enlarged glands must be considered, until the contrary is proved, to be a case of human trypanosomiasis.' Koch[4] confirmed the value of the method in the course of his work, and more recently the British investigators in Uganda have done the same.

As compared with other means of detecting the disease, in the absence of definite symptoms, gland puncture is infinitely the best. This has been shown so clearly and so often that no stress need be laid on it here. While enlargement of the glands does not occur with unfailing regularity in every case, the number of these is so comparatively small that it does not invalidate the practical utility of the method nor the prophylactic measures based on its application.

In obtaining our results, we have used the classification adopted by Dutton and Todd.[5] This schedule is arbitrary, and is one into which the personal equation enters to a large degree, but the exact determination of the class in which the enlarged glands should be placed, e.g., '+—' or '+——,' is perhaps of more academic than practical value. Our figures are based on the palpation of the glands in the posterior triangle of the neck.

In the six months, July-December, 1907, some 9,005 natives were examined, and of these 1,878 had palpable glands, a percentage of 20·85, classified as follows:—

+	+ −	+ − −
5	36	1837

Expressed as percentages of the total number of enlarged glands, we have:—

+	+ −	+ − −
0·26	1·91	97·81

or as percentages of population (based on number examined):—

+	+ −	+ − −
0·05	3·99	23·99

The majority (5,000) were from villages closely bordering the Luapula, but the results from various other districts visited were much the same, so that these figures may be accepted as a fair index of the occurrence of enlarged glands throughout the whole country.

In as many cases as was practicable, gland puncture was performed and the juices thus obtained examined microscopically. The results were:—

Class	Number Palpated	Number Punctured	Number Infected	% of successful punctures
+	5*	5	3	60
+ −	36	30	0	0
+ − −	1837	297	0	0

From this table it will be seen that in the '+—' and '+——' groups the result was uniformly negative, while of the five '+' cases, three harboured trypanosomes.

These findings point to the conclusions that slight enlargement of the glands commonly occurs unassociated with trypanosomiasis, and that excessive enlargement, in practice, means 'sleeping sickness.' By this we mean that such cases should certainly be regarded with suspicion, and should be isolated until puncture can be performed by a properly qualified person. The number of positive cases we have seen is altogether too small to permit of any dogmatic statement as to how great a percentage of '+' glands, in this country, harbour the parasites.

As regards Nyassaland, a report sent to us before leaving England shows that of 3,467 natives examined in various parts of that

* One of these, a child 4 years old, had only one gland, measuring 2 × 1 cm. There was no apparent cause for the enlargement.

territory, 26 had ' + ' glands, 409 ' + —,' and 1,406 ' + — —.' We have no knowledge of any of these having been punctured, so that we are not in a position to say whether any are infected. Judging, however, from our results in Rhodesia, some of the negroes with ' + ' glands might quite possibly be cases of the disease. While these figures are given as the results of the palpation of the glands in the posterior triangle of the neck, one of us was informed by one of the medical men concerned in collecting the figures that glands in all parts of the neck were included. If this is the case with all, the figures would need to be corrected, since some of the glands, e.g., the submaxillary and suboccipital, are very frequently enlarged, from causes other than trypanosome infection.

It therefore seems clear from the relative frequency of slight glandular enlargement, and the uniformly negative findings on puncture, that the axiom 'glands mean trypanosomes' needs to be revised, so far, at least, as Rhodesia and Nyassaland are concerned. It might be stated that excessive enlargement of the glands, sufficiently marked to bring them into the ' + ' category, must be regarded as meaning trypanosomiasis until the opposite has been proved. The practical meaning is that a medical officer is the only person who can satisfactorily apply the method of palpation. This will be considered in more detail below.

OCCURRENCE OF CASES OF TRYPANOSOMIASIS

Three cases of the disease were found in the Luapula division. At the time at which they were first seen, all appeared to be in perfect health, and presented no other signs of the disease than glandular enlargement. In all of them, the glands in the posterior triangle of the neck were the only ones which had increased in size. In the village of one of the cases *Gl. morsitans* was present, but only the one case was found. All had a history of having worked in the Katanga mines some three to four years previously. When it is remembered that human trypanosomiasis is commonly very insidious in its onset, and that cases may remain free from symptoms for years, there can be little doubt that these cases originated in the district mentioned. Contributory evidence that this view is correct is afforded by the fact that no indigenous case was found in any of the

villages along the Luapula, although *Gl. palpalis* is present and the people constantly exposed to their bites. The infection can be clearly traced, then, from Kabinda to Lake Kisale, from there southwards to some of the Katanga mines,* and from these again it has been brought into Rhodesia.

As regards the Northern portions of the country around Mweru and Tanganyika, we have at present no personal experience, but there is reason to believe that cases also exist there. These would not necessarily be introduced from the Katanga although the possibility of this must not be overlooked. Very large numbers of natives from these districts have worked in the mines, and since cases have occurred amongst those who went from the Luapula division, there is just as much reason to expect that cases will be found in all the districts from which labour has been drawn. The other point of introduction would be from the endemic centres along the higher reaches of the Luapula, and from Lake Tanganyika. In 1901, imported cases were present at Moliro,[6] in 1902, at Baudoinville, and within the last year the disease has been reported as being endemic in the vicinity of Vua. As fly exists (*Gl. palpalis*) along the shores of Mweru and Tanganyika, as the people have been communicating freely, and as there have been numbers of Swahili traders crossing from one country to the other with their retinues, cases of the disease might reasonably be looked for. There is good reason to believe that these are present.

All these points bear out in a striking manner the correctness of Dutton and Todd's[6] observations on the way in which the disease has been carried from an infected to a non-infected region.

TRANSMISSION OF THE DISEASE

Wherever sleeping sickness has been found, its distribution has been closely related to that of *Glossina palpalis*. This has been accepted as showing more or less conclusively that the disease can only be transmitted by this species. While it would be idle to ignore the inferences implied by this relationship of the disease and *Gl. palpalis*, there is little foundation for the belief that this fly only is

* At present there are a number of cases of human trypanosomiasis in the hospital at Ruwe, and until very recently, at all events, at Kambove as well.

responsible for the spread of the disease, in the light of our present knowledge.

In parts of the Congo Free State visited by the Expedition of this School to the Congo, the disease was found to be widely disseminated, although *Gl. palpalis* was found only very scantily. Since, therefore, the numbers of this fly did not appear to account for the number of cases, experiments[7] were made with various other biting Arthropods—the larva of *Auchmeromyia luteola* (Congo Floor Maggot), *Ornithodoros moubata*, *Simulium* and Anophelines—to transmit *Trypanosoma gambiense*. All these resulted negatively. The experiments to transmit by means of tsetse flies were also unsatisfactory, and although positive results were obtained, the number of flies required was so great that it was felt the solution had not been reached. In Uganda,[8] the results were similar. Large numbers of flies were required for success, and this only followed when the interval between the 'infecting' and the 'transmitting' feed did not exceed 48 hours. No satisfactory evidence of a developmental cycle of the trypanosome in tsetse flies has yet been obtained, and it can now be accepted as proved that transmission by mechanical means is possible. The importance of this is self-evident. Mechanical transmission does not explain satisfactorily the rapid extension of the disease which has been observed in many instances, nevertheless, whether it is eventually shown that the trypanosomes do pass through a cycle in the tsetse fly analogous to that observed in the case of so many other parasitic protozoa, and that *Glossina palpalis* is the natural transmitter of the disease, the practical importance of this accidental, or mechanical, transmission by other species of the genus cannot be overlooked.

In a report sent in to the Administration of North-East Rhodesia in 1906, Dr. Noble states that Dr. Polidori, of the Congo service, told him that their experience led them to believe that *Gl morsitans* had to be incriminated as well as *Gl. palpalis*. Arguing by analogy from the work on cattle trypanosomiasis, this is what would be expected. As is well known, Ngana is ordinarily spread by *Gl. morsitans* (and probably *pallidipes*) yet successful transmission experiments have been carried out with a Tabanid;[9] and other trypanosomes, naturally transmitted by one species or other of the genus *Glossina*, have been carried from animal to animal by distinct

species of the same genus and such entirely different ones as *Stomoxys*[8] and *Tabanus*. While arguing by analogy is often a fallacious method, some proof that in this particular instance it can be accepted is afforded by the recent demonstration in Uganda that *Trypanosoma gambiense* can be transmitted by *Gl. fusca*.[10]

This question of exactly what species of biting flies, more particularly tsetse, are capable of transmitting human trypanosomiasis is one of the most important which still remains to be decided in connection with this disease. If it can be shown that *Glossina palpalis* is directly responsible for the spread of the disease and that the other species are only accidental carriers, the work of controlling the extension will be very much simplified and the cost greatly lessened. This is a point which merits attention from all the Governments concerned.

In brief, our knowledge as to the transmission of the disease stands thus—

1. The only known method of transmission is mechanical.
2. *Gl. palpalis* and *Gl. fusca* can transmit the disease.
3. At present all other species of *Glossina* must be regarded with equal suspicion.

PROPHYLAXIS

The whole system of prophylaxis is based on the application of gland palpation and puncture. Since by this means we are enabled to detect the disease in its earliest stages in over 97 per cent. of the cases, we are in a position to weed out the infected and isolate them before they have become very dangerous. It is manifest that as long as the trypanosomes are confined to the glands, as opposed to the peripheral blood circulation, the chances of a tsetse fly becoming infected are comparatively small. Koch[11] has also stated that the tsetse flies he employed only became infected when animals were used which had had the disease for a considerable length of time, and in whose blood the parasites were scanty. From this it will be seen that the value of gland palpation is enhanced so far as prophylactic measures are concerned. These measures may be divided into two broad sections—1 major, and 2 minor measures.

1. MAJOR MEASURES.

These are—A. Control of native movements.
B. Segregation of cases.
C. Removal of villages from dangerous zones.

2. MINOR MEASURES.

A. Clearing.
B. Education of the natives.
C. Personal prophylaxis.
D. Destruction of tsetse flies, their larvae and pupae.

1. MAJOR MEASURES.

A. Control of native movements.

Of their own accord, natives do not move about the country in large bands. These are either directly associated with Europeans, Swahili, and Arab traders, or indirectly under their control. Legislation to control the direction of these movements would accordingly do much to prevent the importation of cases from infected to non-infected areas. This legislation should make it an offence, punishable by suitable fines, for any person having infected natives in his employ, taking natives from a non-infected region to an infected one and vice versâ, or otherwise violating the regulations promulgated from time to time with regard to sleeping sickness.* Wherever possible it would be well to have all natives who are travelling for any distance certified as free from symptoms of the disease by a competent person.

In the case of North-East Rhodesia, the only dangerous movements are from Madona to the Katanga mines, and the operations of the Swahili trader along the northern border. We believe that the cases we have found originated at the Katanga mines; in addition there are cases under treatment at Ruwe, and until very recently at Kambove as well; and tsetse flies exist along the whole of the route from Madona to Kambove. The danger of this traffic was pointed out as long ago as February, 1907, and the stoppage of labour-recruiting for the mines was then advised. This, however, was not done until later in the year, when the receipt of reports from Dr. Sheffield Neave, in addition to those previously sent

* That some such legislation is required is shown by the fact that although Madona is now the only place at which the Luapula can officially be crossed, white men have on several occasions crossed the river higher up after leaving that place.

in by Dr. Massey, made it plain that the disease was steadily gaining ground in the southern portions of the Congo Free State. Permission has been given to the Tanganyika Concessions, Limited, to recruit labour in British territory to transport the loads now lying at Madona (6,000 odd), and those actually in transit in the country, to the mines. We are of the opinion that this policy is mistaken, and that the Tanganyika Concessions should be required to take the loads across the Luapula and find the necessary carriers in the Congo Free State.

On the West, Rhodesia is separated from the Congo Free State by a boundary which can be watched with comparative ease, viz., the Luapula river. With exception of fords at the Mombatuta Falls, at Madona and at the Johnston Falls, the river can only be crossed in canoes, and when the river is in flood these fords are impassable. The measures suggested, therefore, to protect the river are the confiscation of all canoes and the placing of patrols at the fords should this be found necessary. To render these measures absolutely effective, the co-operation of the Congo Government will have to be obtained, for if the villages on that side of the river are allowed to retain their canoes, it would nullify to a great degree the benefit derived by the confiscation of the canoes on the British side. If the mining companies in the Katanga refused to give work to any natives of Rhodesia who might get across the river, the general mass of natives would soon learn that it was useless to go to the mines, and the temptation to leave their villages would thus be removed. In addition any uninfected natives of British territory who are in the Katanga at present should be returned immediately.

The operations of the Swahili traders are chiefly confined to the northern border. The obvious way of dealing with them is to refuse licenses and to require them to leave the country absolutely. Regulations to this effect have been passed. The control of a land boundary, especially in a country like Africa, cannot be perfectly effected, but by the stoppage of organised traffic much can be done. Eventually it may be necessary to establish a patrol along the border between Mweru and Tanganyika, but the utility of moving all the villages from a strip parallel with the boundary might first be considered. If practicable, it would do more to stop communication than any system of surveillance, however complete. Here co-operation with the Congo authorities would be advisable.

Another region which requires attention is that part of the boundary between North-East Rhodesia and the Congo State, extending from the Luapula to North-West Rhodesia. One of us was informed by Mr. Croad, the Native Commissioner at Serenje, that the Congo Free State claimed the territory twenty miles to the East of the true boundary, and that in this debated land the Katanga mines are recruiting labour. Until the boundary dispute is settled, the British and Congo authorities should unite in forbidding any recruiting to be done in the country in question.

Along the frontier between North-West Rhodesia and the Congo Free State no natives are now supposed to cross, and as the country along this border is very sparsely populated there is not much violation of this regulation. The most important point is that all loads going to the Katanga mines have to be carried from the frontier by labour recruited in that territory. As we have pointed out above, this should also be the case in North-East Rhodesia.

With respect to Nyassaland, no labour is now allowed to proceed to the Katanga. While this is so, it must not be forgotten that there are there a number of skilled workmen and raw labourers from this country, and these will be returning to their homes at future dates. It is an open question whether some of those who have already gone home have not already carried the infection into the Protectorate, just as has occurred in the case of Rhodesia. In a recent number of the British Medical Journal,[12] we notice that the Principal Medical Officer of Nyassaland does not anticipate the entrance of the disease from the North, and even if this be so, a point on which we still have some doubts, the possibility of it coming in by the route mentioned above must not be ignored. As *Gl. fusca* occurs in the Protectorate, and as it is capable of transmitting the disease, the danger which may result from the introduction of human trypanosomiasis is apparent. The chief line of trade from the North end of Nyassa is from Karonga to Kasama, in Rhodesia. So far as is known at present there is no danger connected with this, though odd cases of the disease, imported from the Katanga, may exist in the neighbourhood. Whether there is any danger of it coming in from German East Africa, we cannot say. Imported cases were present at Udjidji in 1906, and *Gl. palpalis* is found along the German shore of Tanganyika to below Bismarckburg.

B. Segregation of cases.

This is a most necessary precaution. Cases of trypanosomiasis exist in the country and tsetse flies are widely distributed, in one instance, at least, being fairly plentiful in the village in which an infected man was living. The practical application of gland palpation and puncture are the means to be adopted in finding the cases. Dutton and Todd[5] pointed out that any European, or even intelligent native, could apply the method. While it cannot be doubted this is possible, it would be advisable not to rely on the chance assistance of either of these. Even with the best of intentions, the majority of Europeans would not fully appreciate the importance of the issues involved, and would soon tire of practising it. A dependence on results thus obtained would only lead to a false sense of security. Again, the fact that enlarged glands in this country does not necessarily mean trypanosomiasis is another reason why the work should be left in the hands of trained medical officers. The saving of time where palpation and puncture can be done on the spot, and the consequently lessened danger of having possibly infected people travelling through the country to the nearest medical officer, are facts worthy of consideration. At the present date it appears probable that cases are scattered over a wide area of the territory; therefore we would suggest that a sufficient number of special medical officers be appointed to travel systematically through all the villages palpating all the natives and puncturing those in which this was indicated. In the event of cases being discovered, they should at once be removed to a segregation camp for treatment. Before leaving their villages, or, in fact, as soon as the diagnosis had been established, they should be given a full dose of atoxyl, preferably intravenously. This would drive the trypanosomes from the peripheral circulation—Koch[13] states for at least 30 days—and would prevent the possibility of infected persons acting as disseminators of the disease on their way to the camp.

It must not be thought that one visit to a village will be sufficient. Two of the cases we found only escaped earlier detection by reason of the fact that they were absent from their villages when these were first visited.

The districts into which the country is divided for this purpose should not be so large as to make it impossible for the medical officer

to do the work satisfactorily. The villages should be visited at least twice a year, though oftener would be much preferable and advisable. Too much work would be entailed if one medical officer were expected to do all this travelling, and in addition look after a segregation camp. To provide two medical men for each sleeping sickness district would perhaps be too expensive, though if the political divisions (in North-Eastern Rhodesia) are adhered to, this number would be required. In North-Eastern Rhodesia the cases will be found chiefly along the frontier, and it would be quite possible to have one central segregation camp somewhere on the plateau which would serve the requirements of the whole Northern portion of the country. This would have to be under a resident doctor.

It must be admitted that this scheme will entail a substantial expenditure, but the cost will only be a fraction of the loss which would result from a wide-spread extension of sleeping sickness, and in addition there is a fair chance of success if put into operation at once. Another point is that the expenditure would probably not be a permanent drain on the country, as when all the cases have been found and isolated, and when the frontiers have been finally closed, the staff could be reduced. As more knowledge is obtained of the bionomics of tsetse flies, of the fate of trypanosomes ingested by them, and of what flies are capable of transmitting the disease, other methods of controlling its spread may be found.

In Nyassaland the disease is unknown at present, though there is a possibility of it being in the country already.

C. Removal of villages from dangerous zones.

As long as villages are left in places where tsetse flies abound, the introduction of a case of trypanosomiasis is dangerous. We quite recognise that it will be impossible to carry this measure into effect everywhere, but in situations where *Gl. palpalis*, at least, exists, it should be put into application. In other cases clearing must be resorted to. In Rhodesia, this measure would apply to those villages lying along the Luapula, Mansa, Luongo and Kalungwisi rivers and Lakes Mweru and Tanganyika. Whether it can be done in all these situations we cannot say, but with regard to the Luapula, the Mansa and the Luongo it is practicable, and in fact is being enforced. In most cases these particular villages only settled on the river after the

advent of British rule had ensured peace; formerly they were further back in the hills, and it is to these former sites that they are being moved. This procedure will also lessen the chance of natives crossing the river surreptitiously.

2. MINOR MEASURES.

A. Clearing.

In those cases in which the villages cannot be moved, the surrounding land must be cleared. The extent to which this must be done appears to be variable. Dutton and Todd advise 300 yards, while in Uganda, Dr. Hodges[1] states that a break of 50 yards (in the case of *Gl. palpalis*) is sufficient to banish them from their natural haunts. In this connection Madona affords a striking example. On either side of the ferry, the whole shore has been absolutely cleared for a distance of 200 yards. Beyond this again the land is planted with gardens for some distance (about 500 yards to the East and 700 to the West). From the river's edge the clearing extends back for 350 yards, and in the middle of this clearing, some 225 yards from the river, the various residences and offices are placed. The river here is over 400 yards in width. It will thus be seen that the clearing more than satisfies the most exacting demand that has yet been made, but in spite of its extent, specimens of *Gl. palpalis* have been seen and caught on at least half a dozen occasions on the verandahs of some of the buildings, and this too when there was no possibility of them having been accidentally carried from the bushy part of the shore. We would, therefore, consider it more advisable to move villages from fly-infested locations than to leave them in small clearings. The native is notoriously lazy and careless, and even if forced to make clearings, would allow them to grow up again unchecked unless continuously supervised. In cases where villages have to be left, we would insist on the 300 yard clearing as the very smallest that should be allowed.

B. Education of the natives.

The relationship existing between tsetse flies and sleeping sickness should be explained to the chiefs, and the importance of placing their villages in fly-free country. So far as Rhodesia is concerned, most of the natives know from practical experience that cattle and sheep

invariably die when brought into contact with 'tuzembe,' and would therefore appreciate the importance of giving these insects a wide berth. At present sleeping sickness is unknown to the natives, and unless controlled they would not be deterred from pursuing such occupations as fishing, by an abstract fear of a disease of which they know nothing. The ordinary bush tsetse (*Gl. morsitans*) is known to most of them, as mentioned, but they are not so well acquainted with *palpalis*. The chiefs might be asked to notify any case of unusual illness in their villages and to clear the bush around the places where water is drawn. No reliance, however, could be placed on their promises to carry out any regulations.*

C. Personal prophylaxis.

This is obviously directed to the prevention of the bites of tsetse flies. Adequate clothing and the use of some of the means adopted in the prophylaxis of malaria would be applicable here, e.g., head nets where the fly are very bad.

D. Destruction of tsetse flies.

Unfortunately we know of no means of directly destroying them. Very little is known of the bionomics of these insects, so that we are in the dark as to their most vulnerable point. So far as our experience goes, burning the veldt does not make much difference in the number of flies. Indirect measures of banishing them, such as clearing, have been mentioned above.

In brief then, the measures we would suggest for adoption in Rhodesia are—

1. The absolute and immediate closure of the Luapula.

2. The stoppage of transport from Madona to Kambove by natives of Rhodesia.

3. The return of all uninfected natives of Rhodesia, and Nyassaland, from the Katanga mines to their homes.

4. The various mining companies in the Katanga should be requested to refuse work to any native of Rhodesia.

5. The Rhodesian and Congo Governments should refuse permission to recruit labour in disputed territory.

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* For instance, the chiefs along the river have been told that they must not take anyone, white or black, across, yet in spite of this such cases have come under our own observance.

6. All the canoes along the Luapula river should be seized.

7. All Arab and Swahili traders should be expelled from the country.

8. All villages on either side of the Mweru-Tanganyika boundary should be moved back, if possible.

9. Should it be impossible to carry No. 8 into effect, the establishment of patrols must be considered. This would, however, not be so satisfactory.

10. Legislation should be passed to deal with any infringements of the regulations promulgated with regard to sleeping sickness.

11. The Government of the Congo Free State should be requested to co-operate actively with the Administration of Rhodesia in rendering any measures effective which may be adopted from time to time for the protection of the frontiers.

12. That special medical officers be appointed to travel through the country to search for cases of the disease.

13. That a central segregation camp, under a resident doctor, be established in a district free from fly.

14. That all villages be moved, wherever possible, from the vicinity of tsetse flies, more particularly *Glossina palpalis*.

15. In cases where removal is impossible, clearings round the villages at least 300 yards in width should be insisted on.

16. The chiefs should be instructed with regard to the disease, its relationship to tsetse flies, and the importance of keeping the villages in fly-free country.

Madona, *February 1st*, 1908.

In a letter dated Madona, February 13th, 1908, Dr. Kinghorn refers to the official report of Dr. Spillane. . . . . He states that the report verifies what Dr. Todd prophesied in 1906. He also states that *Gl. morsitans*, as a possible transmitter of the disease, is ignored in drawing up preventive regulations.

'Another case of sleeping sickness has been found in the vicinity of Madona. This man, a chief named Matanda, says he has never been in the Congo since Europeans came into the country. His village is not far away from two others in which cases have been found.

'He has been a very big man, over six feet in height and well-developed. Now he is very much emaciated, can only rise when assisted, and walks with difficulty. Has no headache or other symptoms. Glands + — —, and on puncture show trypanosomes. These are also present in the peripheral blood. The case was diagnosed by Dr. Storrs, M.O., and I found trypanosomes in the blood.'

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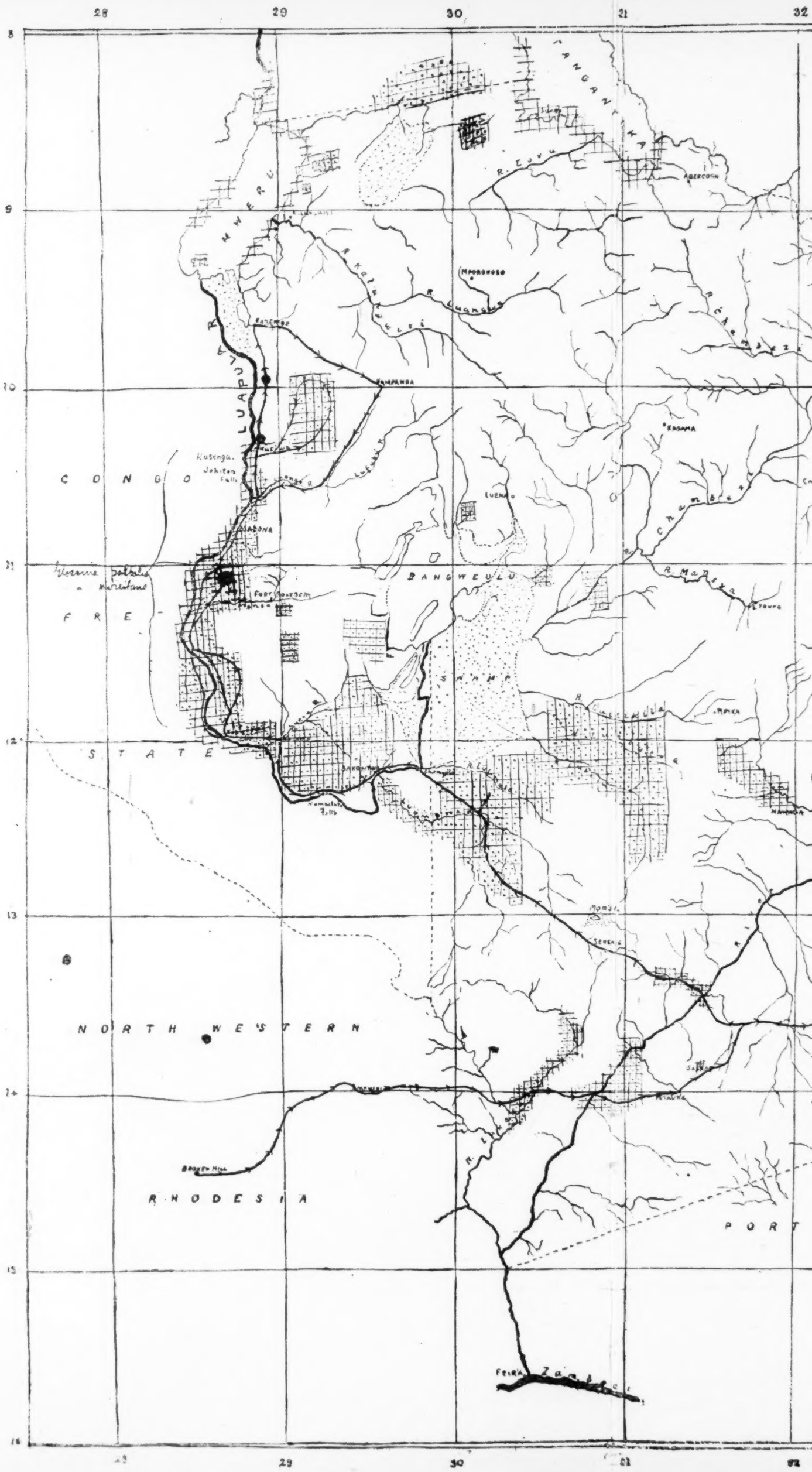
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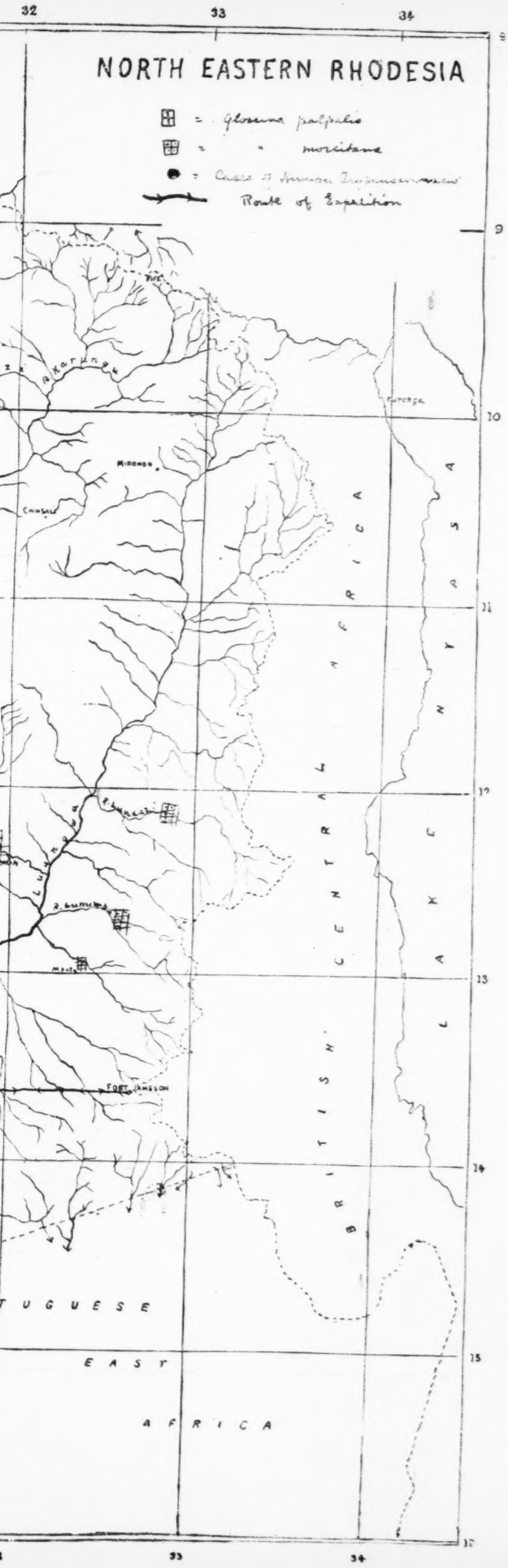
NORTH EASTERN RHODESIA
= Glossina palpalis
= " morsitans
Route of Expedition
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33
34
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15
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Mirongo
Chinsali
Fort Jameson
BRITISH CENTRAL AFRICA
LAKE NYASA
PORTUGUESE EAST AFRICA

A REPORT ON TRYPANOSOMIASIS OF DOMESTIC STOCK IN NORTH-WESTERN RHODESIA

BY

R. EUSTACE MONTGOMERY, M.R.C.V.S.

AND

ALLAN KINGHORN, M.B.

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE EXPEDITION TO THE ZAMBESI, 1907

(*Received for publication* 10 *April*, 1908)

SYNOPSIS

The Expedition reached Broken Hill, the present head of the Cape to Cairo Railway (approximately 14° 35′ S., 28° 40′ E.) on June 17th; on the 23rd the examination of some cattle at a farm in the neighbourhood revealed the presence of trypanosomes.

LOCAL VIEWS ON THE DISEASE

'Fly disease,' 'fly struck' or 'fly' is well known to the inhabitants of the district, and most deaths in cattle are ascribed to it. The views held locally are those of stock owners South of the Zambesi. The passage of cattle through a known tsetse area is only undertaken under compulsion, and wherever possible is effected during the night,

and it is considered that the bite of more than one fly is usually necessary to give the disease.

Cattle are held to be most susceptible, but cases are recorded of animals which do well and breed in villages situated within fly areas. There is a fairly general impression that animals born in the fly area possess a degree of immunity not enjoyed by others, and this is held to account for the herds which some native chiefs are said to own. We have seen such a village near the River Kafue, and have taken *Glossina morsitans* within a mile and a half of it. The chief of this village regards the freedom of their cattle from the disease as being due to care in herding, and admits that if they were taken away from the old garden clearings except at night that sickness would be expected. Another village was frequently quoted as an instance of this inherited immunity, but on arrival there we found that within the past two years most of the cattle had died of fly disease, and the rest, which had been sold to a European, had also succumbed. We only met with one case which would tend to bear out this suggestion of local insusceptibility.

Sixteen head of yearling stock were purchased from a chief near the Kafue in 1902. These animals had been bred there and *Gl. morsitans* is found within half a mile. For three years they were kept on the purchaser's farm, within a few hundred yards of tsetse flies, during which time one animal died suddenly, and six were sold fat for slaughter. In 1905 the remainder were moved twenty miles down the river, and during the rains of 1906-1907 six deaths occurred. Five of these had given birth to calves, and these, with the rest of the cows, appeared in excellent condition when examined in November, 1907. These cattle had spent their lives within a mile of *Gl. morsitans* which had frequently been caught feeding on them. Game is plentiful around. Bushbuck, puku, waterbuck, and hartebeest have often been seen grazing a few hundred yards from the homestead.

The susceptibility of the mule and donkey is disputed. A team of the former and some donkeys were kept for transport near Broken Hill, and are said to have frequently been in contact with the tsetse, but we were not informed of any deaths which could be directly attributed to this cause, and an examination of eight of these mules did not show trypanosomes. We received the histories of three donkeys which are said to have lived in fly areas for from one to three years without ill effect. One of these was later shown to be susceptible to infection by *T. dimorphon*. A second was taken into a badly infested district in August under the belief that it was immune. Its blood was examined in November by a layman, who, however, had a good deal of experience in blood examinations; and

it was stated to have trypanosomes. Fifteen days later it appeared in good health, and we were unable to verify the finding in the single observation made. Dr. Yale Massey showed us films of *T. dimorphon* made from the blood of a donkey which died of the disease at Ruwe in the Congo Free State.

Goats are considered as immune by Europeans and natives alike. This view is held to such an extent that they will graze a herd of these animals over land intended for occupation in order to drive away the tsetse, which is said to be not only harmless to them, but also to be repelled by the odoriferous nature of the adult males. We were informed of many instances where goats had lived and bred within fly areas. From one such area a European purchased forty head in February, 1907. They commenced to die immediately at the rate of one or two a week, the symptoms being emaciation lasting for one or two months. In November three adults (all that remained), each of which had a kid, were examined with negative result.

Native dogs are regarded as immune, and it is stated that natives will expose their dogs, a valuable asset to them, without fear. We have seen three cases of natural infection in 'essential kaffirs.' English and Colonial dogs are considered susceptible; but if born or bred in the fly district their powers of resistance are said to be increased.

No special symptoms are recognised. Gradual and progressive emaciation; periods of manifest depression followed by others in which the animal appears brighter; lacrimation and nasal discharge; these, taken with a history of passage through a fly district, are held to be sufficient for diagnosis. Oedema, enlargement of the superficial glands or paresis, are not mentioned. On autopsy an oedematous condition of the connective tissues and a paleness of the muscles are said to be constant; the presence of fluid in the body cavities has not always been noted by stock owners.

Deaths are said to be more frequent just after the break of the rains, and it is considered that any undue exposure of an animal to water will 'bring out' the latent disease; so much is this held that we have been informed of cattle being purchased subject to a test of pouring a bucket of water over them. Should they not show evident signs of sickness within a few days, they are considered as free from 'fly.' Our observations at Broken Hill, and since, do not

bear out the interpretation of this seasonal prevalence given by owners, most of whom think that the deaths will occur soon after the commencement of the rains irrespective of the date of infection. The more reasonable explanation would be that as waggon transport is impossible except in the dry season, the infection takes place between May and October; and since the disease is of a rapidly fatal character (one to five months), deaths will normally occur about November at the time rains are expected to break. Further, it is obvious that any debilitating influence, and particularly this sudden climatic change, will act as an exciting agent and hasten a fatal termination.

The ordinary method of prevention consists in avoiding tsetse country; where this is impossible, animals are driven through at night, and are kept on an open grass plain during the day, for owners have noticed that the tsetse (*Gl. morsitans*) frequents by choice the bush. A native method of prophylaxis exists, and it appears to have also been used as a curative. Previous to entering a fly area the water of the cattle is restricted so as to compel them to drink a bitter decoction made from the bark of a tree (*Kangomba*) in which the body of one tsetse is placed. At night the animals are kept in a hut and a fire made of the young twigs and leaves of a bush (*m'safwa*), in the smoke of which they remain till morning. The effects of this treatment are held to last for three to four days, but daily adoption is recommended. One European expressed some faith in it, having twice taken an animal into a tsetse zone after fumigation. Another put the matter to a more thorough test, and after medication sent three cattle into a fly area; they all died within six months. As a preventative, fumigation may be to a degree efficacious, for the presence of an obnoxious agent would certainly tend to repel the attacks of the fly; but we question the correctness of the diagnosis in those cases, and they are admittedly few, in which a cure is said to have resulted.

With one exception, cattle owners European and native, have incriminated the tsetse to the exclusion of all other biting flies, and the views common in South Africa regarding the association of *Glossina* and game are also held, but to a more limited and disputed extent. The exception was a native of North-Eastern Rhodesia, whose father, one of the greatest chiefs on the Western side of the

territory, had lost some eighty head of cattle within a year. This man caught us a *Tabanus*, closely allied to *T. dorsivitta*, Walk., and stated that his people believed it to be the cause of the disease. While this is no proof, the spontaneity of the act indicated these natives' belief in the statement, which coming from such a source is the more remarkable, as ordinarily they might be expected to blame the recognised enemy, the tsetse.

DOMESTIC ANIMALS IN THE TERRITORY

These observations refer to that part of North-Western Rhodesia which lies between 28° 40′ East Longitude and the River Kafue, and more particularly to that area in which one of us travelled.

Cattle breeding is extensively carried on by the Mashakalumbwe people, who occupy the North bank of the Kafue as it runs Eastwards to join the Zambesi. Isolated members of the same tribe living to the North of the rest are also cattle owners; the other tribes are not cattle-men, though a few of the largest chiefs whose villages are marked on the map possess a few cows in the kraal. If they own any more, these are quartered out with other chiefs resident in fly-free districts. It would appear that even these few are discontinuing the custom, partly owing to the losses they sustain from the tsetse, and perhaps also because with the presence of Europeans the prices of cattle have risen, and they will no longer replace losses. As practically the whole area North of a line drawn from the South-Western corner of the 'Hook of the Kafue' to the South-Eastern boundary of the Congo Free State is infested by *Glossina morsitans*, it is understood that cattle-raising to any extent would be impossible, and that its absence is not entirely due to the indolence of the native. We are informed that in 'the old days' cattle breeding was extensively carried on here, and that the spread of the tsetse, the epidemic of rinderpest, and the tribal raids that took place before the British entered into possession, have brought about its cessation.

European settlers own one to three spans, each of sixteen to eighteen head, for agricultural work; but the opening up of mines, and the lack of other forms of transport, have caused these animals to be taken from the farms and utilised for this purpose. Cows are scarce, and are now obtained, together with the working bullocks, from the Mashakalumbwe on the Kafue, or from the Barotse Valley

on the Western side of the British sphere of influence, which is said to be free from fly and an excellent cattle country. There are a few animals of the Zebu type coming from German East Africa, or from the Ngoni Country to the East of Rhodesia.

Goats are kept at most of the larger villages, which are relatively few in number. They are of a very inferior strain, small and undersized, and would appear to have been in-bred for generations. Those in the hands of Europeans are somewhat improved, and the rams have been imported from the better stock-raising districts South of the Kafue.

Sheep in North-Western Rhodesia are, so far as we are aware, owned mainly by settlers. In North-Eastern Rhodesia, however, natives own large flocks, mostly small ill-shapen animals, showing but an element of Persian blood. Excepting for the smaller development of their tails, which hang straight at the tip, they are not unlike the Indian *dumbah*.

Equines are limited to a few Europeans. They came from South of the Zambesi, and some of the donkeys from German East Africa.

TRYPANOSOMIASIS IN CATTLE

Our present observations date from June 23rd, between which date and September 30th, 1907, experimental work on the morphology of the parasites and the curative influence of atoxyl and mercury was conducted. Since October we have been travelling continuously.

This work was only rendered possible by the kindness of two agriculturists whose animals were affected—Messrs. J. F. F. Johnson and F. C. Miles—who permitted us to make what use we could of their sick spans. Much of the experimental work on pathogenicity is due to the liberality of the Administration, The British South Africa Company, which, in conjunction with Mr. H. U. Moffat, the Superintendent of the Bechuanaland Exploration Company, placed a sum of money at our disposal for the purchase of the more expensive animals. To the Administrator himself, Mr. R. Codrington, and to Mr. Moffat, it is difficult to adequately express our indebtedness for the constant and continuous interest they manifested in the work. Observations of the effects of the trypanosomes met with upon the usual laboratory animals was only possible

through the kindness and generosity of Dr. Arnold Theiler, C.M.G., the Veterinary Bacteriologist to the Transvaal Government at Pretoria, and of Dr. Robertson in charge of the laboratory of the Medical Officer of Health at Cape Town. Finally, our thanks are due to every European in the neighbourhood for the constant courtesy they extended, and for the many demands made on their time in supplying us with the result of their experiences; to the agriculturists who, without exception, placed their animals at our disposal for examination; and to the various Government Officials who did all in their power to forward the research.

We established a temporary camp and laboratory near Broken Hill, and during our stay there, 36 cases of trypanosomiasis in cattle were detected, of which 29 were under continuous observation. This work was discontinued on September 30th, in order to prosecute our enquiries further North in accordance with the scheme drawn up before leaving England.

The history of many of the 36 cases was uncertain, save that at some period within the past six months they had been in a fly district. One herd, however, possessed peculiar interest as indicating in the most convincing circumstantial manner that biting flies other than *Glossina* can transmit infection. This will be discussed under 'Transmission.'

We have already noted that with the opening up of mines, cattle previously and primarily intended for agricultural work have been utilised for transport, and on *primâ facie* grounds this exposes them to greater risks of infection by bringing them into contact with the tsetse's haunts. At the present time one of the four 'roads' from Broken Hill must be used for waggon transport. The two which run North enter a fly district about fourteen miles out; the third and most frequently used one passes through a patch in which tsetse are generally seen, about eight miles South, but being a narrow zone, night marching may avoid attack. The fourth runs for 112 miles South-West to the copper properties there; only within this last year have tsetse been found encroaching at about the 100th mile. This road joins that coming North from Kalomo, which was used prior to the railway. From the neighbourhood of the junction a road runs North to the copper mines, and is in fly-belts most of the way, and a new one has just been cut in a South-Easterly direction to join the

railway some 30 miles North of the Kafue. This runs most of the way on a watershed; *Gl. morsitans* has not yet been seen, and the nature of the country is not indicative of its presence, but on all the other roads the danger of infection is constant, and when the Northern ones are used it is recognised that all cattle must be sacrificed.

In the first herd examined the trypanosomes seen in fresh cover-glass preparations appeared to be of two varieties. One, the prevailing type also found in other herds, was seen to possess the morphological features of *T. dimorphon*, Dutton and Todd. The second was seen in four of these animals, and later in a fifth coming from another herd, and in two cattle which had been exposed to tsetse-flies experimentally. This, by reason of its extraordinary rapidity of motion in cover-glass preparation, is regarded as allied to *T. vivax*, Ziemann.

INFECTION WITH *T. DIMORPHON*

(1) The natural disease in Cattle.—There is nothing in the clinical picture of this disease to differentiate it in any way from other forms of trypanosomiasis. At some period there is an appearance of emaciation and dulness, the coat harsh and hide-bound, head drooping, eyes dull and watery, but petechiae on the conjunctival membranes were very rarely noted, and occasionally there is a nasal discharge. Weakness or paresis of the hind limbs is not common, and oedema was not seen. Enlargement of the lymphatic glands, notably the prescapular and precrural, is constant, but is of little diagnostic importance owing to its prevalence in apparently healthy animals in which trypanosomes could not be found on blood examination or gland puncture.

All animals under observation were placed at night into the ordinary cattle kraal of the country—an open enclosure fenced to a height of seven or eight feet to avoid the attacks of wild beasts—and were allowed out to graze all day. Temperatures were taken and blood examinations commenced between 8 and 11 a.m., and the temperature taken in the evening between 4-30 p.m. and sunset. For these operations the animals were brought into a kraal-like enclosure leading to a 'crush,' into which each was in turn driven. After the

first day the cattle accommodated themselves to this method, which cannot be held as interfering with the proper thermometric registration. Under these conditions the normal temperature of healthy cattle was found to be approximately 100° to 102° F., two degrees representing the normal diurnal variation.

During the course of the disease the temperature was almost constantly elevated; to a slight degree it was paroxysmal, but, as the accompanying charts show, this was not a marked feature.

Parasites could usually be found on direct examination of peripheral blood. For graphic representation a similar system to that adopted by Lingard is used, but the number of trypanosomes present is so much less (12 to a field (Zeiss Oc IV, Obj. D) was the greatest number seen) that lower values are accorded to each mark. When numerous it was customary to count forty fields and take the average; but when scanty sixty to a hundred, according to indications. No animal is marked '*absent*' unless this latter number was counted; and in necessary cases, as in the treatment experiments, a $\frac{3}{4}$-inch square cover-glass was searched before placing a minus sign.

There is no close relationship between the temperature and the number of parasites seen; sometimes a temperature of 104° or 105° F. was unaccompanied by trypanosomes, or only one to twenty fields; and again, a temperature of 101° has been seen with four organisms to a field. Two cases were observed in which organisms were not seen for two or three weeks, and in one (No. XIII) which was diagnosed on July 18th, trypanosomes were only seen on August 10th (one to a cover-glass during the ten weeks the animal remained under observation). These might be considered as chronic or latent infections, though both died, apparently within the usual time of the disease.

Gland puncture of the prescapular lymphatics was tried as an additional aid to diagnosis, though its general employment is not so generally necessary owing to the fairly constant presence of trypanosomes in the peripheral blood. The method is essentially that described by Dutton and Todd for the diagnosis of human trypanosomiasis, all specimens being sealed with vaseline and examined immediately. It will be seen from the figures below that it is of less value than blood examination as a means of diagnosis, but its

employment is advisable before any suspected animal be considered free from the disease.

	Animals in which tryps. were found in peripheral blood	Animals not showing tryps. peripherally
Number of glands punctured ...	26 ...	33
Tryps. seen in gland juice ...	19 ...	1

From a few observations made in India on camels suffering from Surra we considered that this method was a valuable aid to diagnosis, and in this respect we agree with the views of Dutton and Todd,[4] who used it in the detection of trypanosomiasis in cattle of the Congo; but in Rhodesia our results so far lead us to consider this method as of little diagnostic importance.

The duration of the disease cannot be definitely asserted, but from the views held by local stock-owners and our own observations on animals whose histories are fairly complete, and on experimental cattle, from one to five months would appear to represent the normal. In fourteen of our cases the time which elapsed between diagnosis and death averaged thirty days, and many of these animals were in excellent condition and could not be suspected clinically. It seems probable that in a few cattle a chronic form is established; of this we have no direct evidence, but the histories of some stock examined, and the occurrence of two herds in which a large percentage were clinically cases of trypanosomiasis, but which did not show organisms, would indicate the possibility. In one animal of this nature (No. XXVII) every sign of the disease from a clinical standpoint was shown, but trypanosomes only appeared five days previous to death, after an absence of 15 days.

The lesions observable *post-mortem* are those of an emaciating disease—paleness of the visible mucous membranes, flaccidity and pallor of the muscles. The amount of fluid in the body cavities varied. In some cases it was practically *nil*, whilst in one animal approximately 1750 c.c. were obtained from the peritoneal cavity. Petechiae are present on most of the serous surfaces, notably that of the spleen. The blood clots to a considerable degree, and the white clots common in equines with surra were not seen. All lymphatic glands are enlarged, particularly the precrural, and some of the mesenteric, which are also frequently haemorrhagic. Enlargement

of the spleen is inconstant, but it is usually friable with prominent malpighian bodies, and the capsule studded with petechiae. In only one case, No. XLI, showing marked nervous symptoms, were any gross changes seen in the spinal cord.

Trypanosomes were not constantly seen in the blood at death, nor were films, made direct from the lymphatic glands, invariably positive. Those, however, made from the haemorrhagic lymphatics of the mesentery possessed greater diagnostic value than the others, and owing to the danger of a negative diagnosis being made on a blood film sent in for opinion, we would recommend the forwarding of one made from a haemorrhagic gland in addition, though it must be understood that it is necessary for this to be made as quickly as possible after death in order to avoid undue phagocytosis.

These cattle, compared with those of India, are singularly free from organic changes in any organ, and from other animal parasites. In most (16 in 22) there were a few *Filaria papillosa*, and in many (10 in 22) *Paramphistomum conicum* was seen. *Distomum hepaticum* was not encountered despite the prevalent idea that 'fluke' occurs here. No filarial embryos were seen in the blood, and *Piroplasma bovis* was only seen on one occasion in two animals.

(2) The experimental disease.—The strain of *T. dimorphon* mainly used in these experiments was derived from a naturally infected cow, Case No. XXV. This animal was selected as representative of the disease; as she had been confined to the homestead and had not travelled on the surrounding roads, the danger of a mixed infection being thereby reduced. Though apparently in perfect health, she had, owing to her contact with the sick, been subjected to four examinations between June 26th and July 26th, when she was brought in for daily observation. Trypanosomes were first seen on July 29th, and it is believed that she contracted the disease on the farm. The same strain passed through Case No. XLII served for most of the other inoculations.

1. CATTLE. Both the animals inoculated with virulent blood were later utilised in the experimental work on treatment, which was regarded as of more direct importance. Organisms appeared on the seventh and eighth day following inoculation, and remained constant until the exhibition of atoxyl, in one case, No. XLII, for 24 days. Death in the case of No. XLI was largely due to atoxyl-intoxication.

The other animal is stated to have died suddenly 63 days after treatment was commenced, and 87 days after inoculation.

CASE No. XLI.—August 20th. Inoculated subcutaneously with 2·0 c.c. citrated blood of XXV. Trypanosomes first seen August 27th, one to a cover-glass. Temperature remained but slightly elevated till the morning of the 28th, when it rose to 104° F., and was 105° the same evening. Organisms increased in numbers up to the 30th (three to a field) when 5·0 grm. atoxyl were administered and the normal disease no longer continued.

CASE No. XLII.—August 20th. Inoculated as above. The temperature on the 27th evening was 104° F., and the following morning trypanosomes were seen, 1 in 2 fields. These remained constantly present and in considerable numbers for 24 days. During this period the temperature was between 102° a.m. and 106·2° p.m.; the animal rapidly lost condition, and showed evident clinical manifestations of the disease, and we consider that if treatment had not been attempted death would have occurred within two or three weeks at the outside. (vide chart i.)

Two other cattle, Cases Nos. XLIV and XLV, which were infected by exposure to the bites of *Glossina morsitans* are described later under 'Transmission.'

2. DONKEY.

CASE No. LV.—This animal is said to have lived in fly districts for upwards of three years and was considered immune. September 3rd, inoculated subcutaneously with 2·0 c.c. citrated blood of XLII. During the four weeks it remained under observation there was no change from normal in its temperature and daily direct examination of blood and three centrifugal examinations did not reveal organisms.

On November 20th it was again examined and *T. dimorphon* found, three to a cover-glass. It did not at this time show any clinical indications of the disease. Since that date we have not received any report as to its condition.

3. SHEEP. The disease in these animals and goats is of considerable interest, for in the quite characteristic temperature chart we have evidence for distinguishing this trypanosome from that which we hold resembles *T. vivax*, Ziemann. Three sheep were inoculated; in two healthy animals the incubation was seven and eight days, in a third, which had passed through a previous infection by the other trypanosome, it was eleven days.

In both healthy animals the temperature assumed at first the type produced by the benign tertian form of malarial parasite in man, later giving place to the quartan form. The regularity of the fever is striking, as also is the fact that each exacerbation was accompanied by an influx of trypanosomes into the peripheral circulation. In order to demonstrate this periodicity of trypanosomes and temperature, these animals were examined every three hours for a period of 56 hours, for as we are unaware of any other form of trypanosomiasis in which this feature is so pronounced, an effort was made to ascertain, if possible, any developmental cycle undergone by the

parasite in the sheep. The time at our disposal, however, was not sufficient for a careful study of the question, and our observations were without definite results.

No special symptoms were noted. During the course of the disease a rapid emaciation took place, but all the animals were alive when the work was concluded at the end of September. Each access of fever was manifested clinically by the weakness, depression and excessive lachrymation shown by the animal. Oedema was not seen in either these animals or goats.

Parasites were never numerous: on one occasion only were as many as six to a field seen. The number, however, depends upon the period in the onset or decline of the paroxysm at which the routine examination happened to be made.

CASE No. XXXVI.—An aged ram, fat-tailed variety, purchased locally, but probably imported from the South. August 7th, inoculated subcutaneously with 5·0 c.c. citrated blood of XXV. The temperature became irregular on the 5th, and organisms were first seen on the eighth day, August 15th. For the first fourteen days the paroxysms were tertian in type, afterwards becoming less regular and approaching the quartan type. Emaciation was rapid, but the animal was still alive on November 20th, and organisms were present. (vide chart iii.)

CASE No. LVI.—Two-year-old female. September 3rd, inoculated subcutaneously with 1·0 c.c. citrated blood of XLII. Organisms first seen on the 7th day, September 10th, and the initial thermal paroxysm occurred during the night of the 11th. On the evenings of September 14th, 16th, 18th, 20th, 22nd, and 24th, the temperature exceeded 106° F., and on the 28th it reached 107°. During the twenty-one days of observed disease the type was essentially the same as in No. XXXVI, the chart of which is reproduced.

CASE No. XXXIII.—Male, aged one year. Between August 19th and September 2nd this animal had shown what we regard as *T. vivax* with which it had been inoculated. After this latter date the temperature remained about normal and trypanosomes were not seen.

September 17th, received subcutaneously 0·5 c.c. heart blood of guinea-pig, Case XLVI, dead with *T. dimorphon*. The temperature was faily constant until the 27th, when it commenced to rise. On the 28th, the 11th day, *T. dimorphon* was seen, the first paroxysm occurring the same day, the second (107·2°) on the evening of the 30th, the last day of observation. (vide chart ix.)

4. GOATS. These animals, as already stated, are regarded as immune. Three readily took infection with *T. dimorphon* after an incubative period of seven to twelve days. The disease is of essentially the same type as that in sheep, viz., a tertian and quartan febrile reaction and a concomitant influx of organisms into the peripheral circulation. No special symptoms were observed.

CASE No. XXXV.—Male, aged one year. August 7th. Inoculated under the skin with 5·0 c.c. citrated blood of XXV. The temperature became irregular until organisms appeared on the seventh day, August 14th. During the seven weeks

of disease in which this animal was observed, paroxysms occurred with the regularity of those in Sheep, only the thermal reaction being more pronounced—108·2° F. on one occasion. Emaciation was rapid, and the animal died in the interval of one and a half months that elapsed previous to November 20th. An exact date was unobtainable as no European had been on the farm. (vide chart iv.)

CASE No. LVII.—Male, aged two years. September 3rd. Inoculated subcutaneously with 1·0 c.c. citrated blood of XLII. Organisms were first seen on the ninth day, September 12th, and paroxysms occurred on the 15th, 17th, 18th, 20th, 22nd, 24th, 26th, 28th, and 30th. (vide chart v.)

CASE No. XL.—Young male. Inoculated on August 9th with blood of Goat, No. XI, containing *T. vivax*. Trypanosomes were never seen, and on September 17th it received 0·5 c.c. heart blood of guinea-pig, No. XLVI, dead of *T. dimorphon*.

On September 29th, the twelfth day, *T. dimorphon* was seen, and again the following day the last of observation. On November 20th parasites were still present, and the animal had lost much of its condition.

5. DOGS. Three native dogs were inoculated with this strain of *T. dimorphon*, and despite the suggested immunity of the race, all took the infection readily, and we shall later note that naturally infected native dogs were met with, in which, however, the parasite did not show the morphological characteristics of *T. dimorphon*.

In all three, the disease was acute; death taking place within two weeks of the appearance of organisms in an adult, and within ten days in young animals of three months old.

Emaciation is rapid; the coat becomes harsh, eyes lachrymose, and in the adult, opacity of the cornea was noted three days before death. Superficial lymphatic glands, notably the prescapular, become enlarged and soft, but not painful; a feature, however, noted in certain apparently healthy dogs. Only one gland puncture was made in the case of an adult, when organisms were not seen in the peripheral blood; but its employment did not reveal trypanosomes. Nervous symptoms were noted in one young dog, No. LIV, which showed partial incoordination of the hind limbs on the day preceding death. The incubation period was sixteen days in adult, and seven and eleven respectively in young animals. The temperature curves of these latter are most irregular; hardly ever elevated, but showing a tendency to become sub-normal. In the older dog the type is that of other trypanosomiases, showing a close resemblance to many charts of canines infected with *T. evansi*. Organisms increased progressively up to death. In the adult the temperature and trypanosome curves synchronised.

CASE No. XXXIV.—A 'kaffir' bitch, aged about two years. August 7th, inoculated subcutaneously with 1·0 c.c. citrated blood of XXV. The temperature

remained normal until the morning of the 21st when it commenced to rise, continuing to do so gradually till the morning of the 26th when it was 105° F. That evening it was 102°, and it kept normal for three days to rise again for the second paroxysm on the 30th. Trypanosomes appeared on the third day after the temperature rose, August 23rd, one to a cover-glass, and increased progressively to the 25th, one to six fields. On the 26th, 27th, 28th, they were not seen; they reappeared on the 29th, and were twelve to a field on September 1st. They gradually fell in number towards death, which took place on the night of the 4th. (vide chart vi.)

CASE No. XLIII.—A 'kaffir' pup, aged three months. August 20th, inoculated 0·5 c.c. citrated blood of XXV. The temperature was very irregular throughout, varying from 96·4° to 103·8°. Trypanosomes appeared on the seventh day, and remained constantly present till death on the fifteenth day. During the last three days they were very numerous, ten to a field, and the temperature was usually subnormal.

CASE No. LIV.—Brother to the last. September 8th, inoculated subcutaneously with 1·0 c.c. citrated blood of XLII. The temperature showed considerable daily variation. Organisms appeared on the eleventh day, September 14th, one to five fields, and remained present in slightly increasing numbers, till death on the eighteenth day after inoculation, September 21st.

Post-mortem appearances.—In all three animals the most noticeable feature was a considerable enlargement of the spleen, which in the adult dog (weight about 30 lbs.) measured 33 cm. in length, 10 cm. in width, and 4·5 cm. in thickness; dark in colour, sofe and friable and edges rounded. Lymphatic glands of the mesentery enlarged congested and haemorrhagic. Petechiae studded the serous membranes; the liver pale and friable. The pericardial sac contained from 20 to 75 c.c., and the peritoneal cavity of Case LIV 250 c.c.; the amount in the thoracic cavity was not greatly increased.

6. RABBIT. Only one animal was inoculated. The disease in this was of chronic nature, and organisms were rarely seen in an ordinary cover-glass examination.

CASE No. XXXVII.—August 7th, inoculated intraperitoneally with 1·0 c.c. citrated blood of XXV. During the four weeks during which it was taken, the temperature showed no gross variations. Organisms appeared on the tenth day, one to a cover-glass, and on the following day five to a cover-glass were seen. They were again detected on the fourteenth, fifteenth and thirty-second day after inoculation; from this time they were not seen on the daily examination up to September 30th, nor on those made about every fifth day since. During the first month of disease the rabbit lost condition; hair began to come out at the base of the ears, around the eyes, and on the rump, and sores formed on both tarsi. No oedema was noted, nor did any opacity of the cornea or signs of paralysis occur. During the next three months the rabbit appeared to improve in condition, and the tarsal ulcers dried up. Towards the last fortnight a slightly purulent discharge collected on the nostrils and around the eyes. Death took place in a very emaciated state on January 21st, 1908, 168 days after inoculation.

Post-mortem.—The spleen was considerably enlarged and rounded, but firm in consistency, the mesenteric lymphatics swollen but pale. Trypanosomes could not be found on direct examination; no sub-inoculations were made.

7. GUINEA-PIGS. Five guinea-pigs were inoculated with this trypanosome. In all, the disease was of a rapidly fatal character, the

average duration after appearance of organisms being only twelve days. The incubation period in three cases was nine days; in the other two, sixteen and nineteen respectively. Organisms were constantly present after detection, and were numerous up to death, which occurred ten to fourteen days later.

The temperature in the one animal taken varied little from normal until the trypanosomes appeared, when it rose and continued slightly elevated to death. The loss in condition was rapid, and the visible mucous membranes became very pale. Conjunctival discharge was not a constant feature, and in no case was any indication of paralysis noted.

On *post-mortem*, the spleen was much swollen, measuring from 5·5 to 7 cm. by 3·5 to 4 cm., congested, rounded, soft and friable, with an average weight of 14 grammes. In one case rupture of the capsule had accelerated death. The lymphatic glands were enlarged, oedematous and congested.

9. WHITE RATS. Five of these animals were inoculated. The period of incubation varied between six and ten days, and trypanosomes remained constantly present till death, which occurred in from 18 to 29 days after inoculation.

On autopsy, the spleen was enlarged, congested, soft and rounded, and the minute mesenteric lymphatic glands were distinct and haemorrhagic.

INFECTION WITH *T. VIVAX*

At the examination of the first herd on June 23rd, we noticed in one bull the presence of an organism, whose extraordinarily rapid passage from edge to edge of the cover-glass, and the transient corpuscular displacement produced, caused us to consider it as a spirochaete. When our camp was established this animal was brought under daily observation, and stained films, and later, cover-glass preparations some hours old, showed the organism to be a trypanosome. In the same herd one other animal was seen to be similarly affected, and two in which *T. dimorphon* had been found showed an occasional parasite whose rapidity of motion approximated to that of the bull. Three days before leaving Broken Hill we examined 14 cattle not previously inspected, one of which showed the same organism.

(1) Natural infection.—It would appear that stock-owners do not recognise more than one form of 'fly disease,' and that the animals infected by this trypanosome are considered as cases of ordinary fly disease. Of these five animals, that first seen was kept under observation for ten days at the original camp, and was later purchased and brought to that at which our experimental work was conducted.

Both animals which showed a mixed infection died within three weeks of diagnosis. The one detected just before leaving Broken Hill was alive, but considerably thinner six weeks later; whilst the fifth was reported to have died in August, seven weeks after parasites were seen, at which time it was in good working condition.

CASE No. VIII.—Bull, aged about five years. (vide chart vii.) No very reliable history of this case is available, but it appears that this animal, with the rest of the herd, had been grazed away during the rainy season of 1906-1907, and had passed through fly areas on the way home in February, and that since that date it had gradually lost condition.

On examination, it was emaciated and hide-bound, signs of excessive lachrymation were present and the conjunctivae showed a few small petechiae, a condition seldom noted in animals infected with *T. dimorphon*. Prescapular and precrural glands enlarged; abdomen tucked-up; no oedema, but the bull presented all signs of trypanosomiasis, from which it was suspected of suffering by the owner. The case was under observation for 26 days between July 10th and August 15th.

A noticeable feature in the temperature is the great daily variation, as much as 5·4° F. being met with. By joining up the evening or morning registrations the 'curve' produced would not be great; there is no suggestion of a paroxysm, and the mean of morning and evening temperatures would not vary much from normal.

Trypanosomes are always scanty, from one to ten to a cover-glass was the usual number, only exceeded on four occasions, when approximately one in ten fields was seen. The great rapidity of the organism rendered any degree of accuracy in counting impossible, for their presence was generally only ascertainable by the slight displacement of corpuscles which accompanied their hurried passage across the field, a passage it was impossible to follow by any ordinary movement of the slide. With such an organism it is very probable that the one or two organisms present in a preparation would not come within the field of vision. On two occasions when they were not seen, centrifugalisation showed them to be present, and in one case a trypanosome was found in a thick-stained film. We would consequently suggest that the trypanosomes were almost constantly present in the peripheral blood and that with this parasite there is no great paroxysmal increase, and their detection is to a more considerable extent a matter of chance than is usual in the other forms of Trypanosomiasis.

During the 25 days of observation, parasites were seen on 16 occasions, eleven were marked +, one ++, and four +++, three of these latter occurring in the last week of life, and the other after the animal had been driven 22 miles in two days to the camp. Two days previous to death extreme weakness was manifest, the patient falling down several times and showing great difficulty in rising.

Post-mortem commenced 15 minutes after death. Rigor mortis distinct, mucous membranes pale, and a few petechiae on one conjunctiva. Skin closely

adherent, subcutaneous tissues firm and dry. Muscles pale but firm. *Thorax.* Only a few c.c. of a straw coloured fluid present. Lungs normal, save for slight emphysema in both apices. Heart normal in size; no fat in auricular-ventricular furrow; muscular tissue pale and streaked with fatty degeneration. Blood dark with half-formed clots in each chamber. *Abdomen.* Approximately 50 c.c. faintly blood-tinged fluid. Serous membranes show a few petechial haemorrhages on both parietal and visceral surfaces. Fat practically absent. *Liver* pale, slightly fatty and friable. Gall-bladder distended, bile of normal colour and viscid. *Spleen,* not enlarged. Capsule firm, somewhat fibrous and studded with small haemorrhages. Malpighian bodies pale, the rest of the splenic pulp pale, lying within the unduly defined trabeculae. *Kidneys* pale, capsule strips readily. Other organs normal. *F. papillosa and P. conicum* present.

(2) The experimental disease.—The organism for this work came from the bull No. VIII, whose history has just been given, and from several animals inoculated from it.

1. CATTLE.

CASE No. IX.—Calf aged nine months. (chart viii.) July 11th, 1907, inoculated subcutaneously with 10·0 c.c. blood direct from VIII. This animal was under observation for ten days after inoculation, during which time the temperature remained about normal and parasites were not seen. Daily examination was recommenced on August 2nd, when it arrived at the new camp.

Trypanosomes were not seen on arrival, but appeared the following day, and as with case VIII were intermittently present until death, which occurred 61 days after inoculation. During the 38 days following detection of organisms, these were marked + on seventeen, ++ on four, and +++ on four, and on thirteen occasions they were not seen. Two days before death, when the animal was very weak, they were extremely numerous, almost swarming across the field, but still retaining their great motility.

The temperature chart is of the same nature as that of the bull; daily variations of 3° to 5° F., without paroxismal tendencies except during the first week after arrival at the new camp, which might possibly be that accompanying the initial influx of organisms.

The loss in condition was gradual, but not excessive, and to the end the animal presented a bright appearance despite a progressive weakness, marked during the last few days. There was but little lachrymation, but petechiae on the conjunctival membranes were observed on several occasions.

Prescapular and precrural glands were enlarged at the time of inoculation, and did not show any perceptible increase in size.

The animal was accidentally killed by a leopard.

2. DONKEY. One animal only was inoculated, and during the twenty-four days of observation organisms were not seen.

CASE No. LVIII.—September 6th, inoculated subcutaneously with 2·0 c.c. citrated blood of IX. The temperature rose to 103·2° on the evening of the same day and the animal showed slight abdominal distension and pain. It was again 102° on the evening of the sixth day, and the donkey was somewhat dull with a watery discharge from the eyes. It appeared well the following day, and showed no further symptoms up to September 30th. During this time no trypanosomes were seen, nor did they appear present on November 20th, when the animal still retained a healthy look.

3. SHEEP. Four sheep were inoculated with this trypanosome, and in three the organism reappeared. Only one, a small weakly animal, died during the period they were under observation; and from an examination of the temperature charts and of the animals themselves, it would seem that recovery may occur naturally, though, of course, we were unable to prove this in the time. The inoculation period in two cases was seven days. Trypanosomes were always scanty, rarely exceeding four to a cover-glass, their activity being as great as in cattle. This fact may account for the irregularity of their detection and the apparent lack of relationship between their occurrence and a thermal rise. The picture presented by the temperature chart differs absolutely from that following inoculation with *T. dimorphon.* The temperature rose between the fourth and seventh days, and fell again a few days later, and remained irregular for two or three weeks. There was no periodic exacerbation of a tertian or quartan type.

CASE No. X.—A small 'weedy' animal of five months old. July 11th, inoculated subcutaneously with 5·0 c.c. blood direct from Case No. VIII. The temperature rose on the fourth day and continued elevated for the ten days it was under observation at the first camp. Mr. Johnson, on whose farm the second camp was established, kindly visited the animal nine days later and made films in which trypanosomes were detected. It was then weak, and the native in charge said it had been sick for three days. It was carried in, 22 miles, to our new camp and died almost immediately on arrival.

Post-mortem.—Trypanosomes were not found in the blood or gland juice. Visible mucous membranes pale, those of the conjunctivae showing a few petechiae. The subcutaneous lymphatic glands were enlarged, those of the mesentery being also haemorrhagic. There was no excess of fluid in the body cavities. Small petechiae studded the pleurae and pericardium. The spleen was not enlarged, its capsule was firm and showed a few petechiae. The liver and kidneys appeared normal. Neither intestinal parasites, nor *D. hepaticum* were found to account for the debilitated state at the time of inoculation. Two specimens of *Cysticersus tenuicollis* were present in the mesentery.

CASE No. XXXIII.—A healthy sheep aged one year. August 7th, inoculated subcutaneously with 1·0 c.c. citrated blood of IX. The temperature rose to 105·2° on the morning of the sixth day and organisms were seen the next morning. During the succeeding six weeks the temperature was four times above 105°, and trypanosomes were seen on eleven occasions. After September 2nd the temperature remained about normal and parasites were not observed. On September 17th it was inoculated with *T. dimorphon.* (*vide antea* chart ix.)

CASE No. XXXIX.—A healthy sheep aged one year. August 9th, inoculated under the skin with 1·0 c.c. blood direct from goat, Case XI. Trypanosomes appeared on the seventh day, and for the next three weeks were fairly constantly present, but during the last three and a half weeks of observation they were only seen seven times. The temperature rose on the sixth day, and during the ensuing forty days of disease was similar to that of bovine piroplasmosis in India.

CASE No. LIX.—A companion sheep to the last. September 5th, inoculated subcutaneously with 1·5 c.c. citrated blood from IX (parasites present). The temperature remained normal and trypanosomes were not seen during the twenty-four days it continued under observation.

4. GOATS. Three goats were inoculated, one becoming infected. Parasites were intermittently present in scanty numbers in the one positive case.

CASE No. XI.—A goat aged one year, in poor condition and coming from a herd infected with scabies. July 11th, inoculated subcutaneously with 5·0 c.c. blood from Case VIII. Organisms were not seen during the ensuing ten days. It arrived at the new camp on August 2nd, and trypanosomes were first seen on the 5th of that month. They continued intermittently present in scanty numbers for the six weeks of life remaining.

The temperature, as in sheep, showed no tendency to reproduce the malarial type, but assumed rather that seen in the two infected cattle.

This animal became badly infected with a form of dermatitis, which spread to the lips and around the eyes and ears, causing considerable irritation and probably hastened death, which occurred on September 13th, the sixty-fourth day after inoculation.

Post-mortem.—Trypanosomes were not seen in blood or gland juice. Mucous membranes pale, a few petechiae on the conjunctivae. A few small circumscribed ulcers were present on the gums and hard-palate. No fluid in the thoracic and abdominal cavities. Heart pale and flabby, lungs normal. Liver pale but of firm consistence, capsule adherent to the diaphragm, gall bladder distended. In the large biliary canals several tape-worms were found. Spleen showed no gross alterations. Mesenteric glands pale and not greatly enlarged. Small concretions resembling those formed by *Œsophagostomum columbianum* occurred in the large intestine. (vide chart x.)

CASE No. XXXII. Healthy goat aged four months. August 7th, inoculated subcutaneously with 1·0 c.c. citrated blood of Case IX. The temperature was irregular, but trypanosomes were not seen, and it was alive and in good condition on November 20th. Sheep, Case XXXIII, inoculated at the same time became infected.

CASE No. XL. August 9th, inoculated with 1·0 c.c. blood direct from goat, Case II. The temperature showed a daily variation somewhat greater than usual, but trypanosomes were never seen. On September 17th it was inoculated with *T. dimorphon* and became infected on the twelfth day. Sheep, Case No. XXXIX, inoculated on August 9th from the same Case XI became infected.

5. MONKEY. An adult female, probably *Cercopithecus pygerythrus*, was inoculated subcutaneously on September 6th with 1·0 c.c. of blood from Case IX. It remained active and in perfect health until October 1st, when it died suddenly through the maltreatment of the boy in charge.

6. GUINEA-PIGS. Five guinea-pigs were inoculated from, respectively, Cases IX, XI, XXXIII, XXXIX and X (*post-mortem*). They showed no disturbance in health, the temperature remained normal and organisms have never appeared. They are all still alive,

February 18th, 1908, despite the rough usage to which they have been subjected on the march.

7. DOGS. Three 'kaffir' dogs were inoculated from Cases IX and XXXIII. The temperature remained normal, and organisms were never seen. One appeared to lose condition during the two months following inoculation, but later regained it, and with the others is now alive.

8. RABBIT. A rabbit was inoculated on August 3rd with 1·0 c.c. of blood from Case IX, when parasites were present. Organisms were never seen, and it continued in perfect health until November 14th, when it suddenly died. On *post-mortem* no signs of trypanosomiasis could be found.

9. WHITE RATS. Five white rats were inoculated simultaneously with the five guinea-pigs. None have shown any disturbance in health, and four are still alive. The fifth was inoculated in November with a dog trypanosome, and died.

INFECTION WITH *T. THEILERI*

During the routine blood examinations of cattle Nos. XVI, XXI and XXII, a large trypanosome was seen on one occasion in each. Further examinations in fresh and centrifuged specimens were negative. In Case XVI this trypanosome was seen three days after *T. dimorphon* had been expelled by means of atoxyl, and four days before its reappearance.

CASE No. XXIII. A bull naturally infected with *T. dimorphon*. August 2nd. Inoculated subcutaneously with 90·0 c.c. citrated blood of Case XXII, which showed *T. theileri* that morning, *T. dimorphon* also present. The animal died of *T. dimorphon* infection three weeks later without ever showing *T. theileri* again.

TRYPANOSOMIASIS IN SHEEP (*T. DIMORPHON*)

A European who visited the camp informed us of mortality amongst his sheep, which were kept in a camp on the Lukanda river, 45 miles North of Broken Hill. No specific details were obtainable, but it appears that between 30 and 40 had been sick and had died or been destroyed. *Gl. morsitans* exists all round, but infection by *D. hepaticum* was suspected. A visit was paid to this camp on November 23rd, during the owner's absence, and three sheep found alive. One was *in extremis*, and blood examination revealed the presence of *T. dimorphon*. On *post-mortem*, there was an excess of fluid in all cavities, the lymphatic glands much swollen and oedematous but pale, the spleen was enlarged, soft and rounded.

NATURAL TRYPANOSOMIASIS IN DOGS

We saw three dogs which were suspected by their owners of having naturally acquired trypanosomiasis. All had, at some recent date, been in the fly country around N'dola, 15° S., 24° 40′ E., and on examination showed trypanosomes and the clinical indications of the disease—a haggard appearance, anaemia, loss in condition, and in two, corneal opacity.

Sub-inoculations were made from two of these dogs.

From Dog 'A.'

1. GUINEA PIG.

CASE No. LXXII.—Nov. 8th, 1907, inoculated intraperitoneally with 1 c.cm. citrated blood. Parasites first seen on Nov. 20th and at each succeeding examination, about every fourth or fifth day, up till death on January 18th, 1908. Paralysis of the hind legs was noted on December 25th, but the animal recovered in three days. The same symptom reappeared on January 16th, and continued up till death two days later. Duration of disease 72 days.

2. RAT.

CASE No. LXXIII.—Same date. Inoculated subcutaneously with 1·0 c.cm. citrated blood. Organisms appeared on November 18th (tenth day) and were present at each examination up to January 10th, when the animal died. Duration of disease 64 days.

From Dog 'B.'

1. GUINEA PIG.

CASE No. LXXVII.—November 15th, 1907, inoculated intraperitoneally with five drops of blood in citrate solution. Organisms appeared between November 22nd and 26th, on which later date they were present in fair numbers. On one examination, made December 12th, they were not found but were present on all other occasions. Rat died January 12th. Duration 68 days.

2. RAT.

CASE No. LXXVI.—Same date. Inoculated intraperitoneally with five drops of citrated blood. Parasites appeared between the 26th and 29th of November, and were constantly present on all succeeding examinations up to January 3rd, when it died—49 days. This rat had previously been inoculated with blood containing *T. vivax*, but had not shown infection.

I. MORPHOLOGY OF CATTLE TRYPANOSOMES

T. DIMORPHON, Dutton and Todd

The parasite commonly encountered in the naturally infected and the experimentally inoculated cattle corresponds to the short forms of *T. dimorphon* described by Dutton and Todd as 'tadpole' and 'stumpy.' The former predominated in all animals, except during

the last few days of life in a few cases, when the stumpy type was more frequently seen.

If the distinction between 'stumpy' and 'long' is based upon the possession, by the latter, of a definite flagellum, this was not encountered in cattle. Its appearance, however, in sub-inoculations indicates that the parasite in question belongs to the *dimorphon* group. 'Tadpole' forms were found in every animal, with the exception of one moribund case mentioned later. They measured from 9·75 to 15·3 μ in length, and up to 1·5 μ in width. The body protoplasm stains a rather deep blue with the Giemsa stain, granules rarely being present; blepharoplast terminal or sub-terminal, small and rounded; nucleus a short oval, 1·7 to 2·5 μ in length, staining homogeneously a rather dark purple. Undulating membrane very rudimentary; in some, a small, fin-like, single fold could be seen. Flagellum absent, but an anterior prolongation of the protoplasm occasionally supported a minute extension of the rim of the undulating membrane. The posterior extremity is usually bluntly angular, but every gradation was met with. The greatest width lies posterior to the nucleus. Forms slightly larger than the above, which may be classed as 'stumpy,' were occasionally seen in some animals, particularly in those in which the disease was running a more acute course, and have been constantly observed, but they become relatively more numerous towards death. In one animal, which had not shown organisms on two examinations previously, only this form was found at a third examination when the beast was moribund. It was not constantly to be observed at death; in five animals none but what are termed 'tadpole' forms were seen. This form measured from 17·75 to 21·25μ, and is of a stouter build than the 'tadpole.' The protoplasm assumes a pinker tint, the blepharoplast is better defined the nucleus almost invariably distinctly rounded and staining less deeply, and the widest part of the body is more usually on this level. An undulating membrane could be more easily distinguished, in some rare cases two or three folds being visible, and the bordering rim is commonly produced with the anterior extremity of the body to form an abruptly square-cut bristle-like beak, not exceeding 2μ in length, but prominent in a well-stained preparation. Granules were seen in some, and divisional forms of this type were encountered. The distinction between this form and what is described as 'tadpole' is

more marked than between it and the 'long' form. These latter, that is to say, a trypanosome possessing a flagellum of more than a few μ in length, were not seen in cattle, and were only found in inoculated guinea-pigs and rats. In these animals they measured from 25 to 31 μ in length, and could, on the whole, be clearly separated into the two arbitrary classes, 'male' and 'female,' the former being thinner, staining more homogeneously pink, the nucleus elongated, and the protoplasm free from granules; the latter stouter throughout the whole length, the nucleus oval, and the protoplasm taking a blue tint and often containing granules. The flagellum measured from 5 to 11 μ, and continued from an undulating membrane which was not always so evident as in the larger of the 'stumpy' forms. The blepharoplast is fairly prominent, rounded or oval, and commonly situated some little distance from the posterior extremity, which itself assumes most frequently a rather finely tapering point. The width of these forms varied between 1·5 and 2·5 μ, dividing forms being as much as 3·5 μ across. While we refrain from the use of the expression 'free flagellum,' there appears little doubt that in the larger of the 'long' forms, such a structure, free of cytoplasm, does exist. In the large 'stumpy' and the short 'long,' which cannot be clearly separated, the cytoplasm is unquestionably continued for a certain distance anterior to what we would regard as the normal extremity of the body, the place where body, undulating membrane and flagellum meet, and it is often impossible to determine the point at which this prolongation ceases.

In fresh cover-glass preparations the corpuscular displacement is local and the organism does not readily pass out of the field of the microscope. The smaller forms cause, naturally, less commotion amongst the corpuscles. They are more readily retarded by these bodies, and the slow rolling displacement they produce is markedly different from the furious lashing of those with a long flagellum and the resulting scattering of the corpuscles. Neither form was ever noted to produce the peculiar 'catherine-wheel' effect seen in blood containing *T. vivax*.

We were unable to make permanent slides from our donkey during the visit of November 20th, but Dr. Yale Massey kindly allowed us to examine the slides he had made on the day previous to the death of a naturally-infected donkey at Ruwe, in the Congo to the North of the

Rhodesian district we have under review. Short and long forms were present in this animal, the classification of 200 being 'tadpole' 10 per cent., 'stumpy' 70 per cent., and 'long' forms 20 per cent. The first-named measured between 10·5 and 14·75μ, the 'stumpy' from 15 to 18·25μ, and the 'long' forms between 18 and 27·25μ. In some of these latter, a flagellum measuring from 3 to 5μ, which appeared quite free of cytoplasm, was seen. The structure of these several forms was essentially the same as that of those already given.

In sheep and goats only the 'tadpole' forms were seen. These measured from 9·75 to 14·5μ in length and from 1 to 1·75μ in width. In no particular could they be held to differ from those in bovine blood, and in the films examined only one form was seen which might possibly be regarded as 'stumpy.' It has been noted that none of these animals died whilst under observation, and this may account for the non-detection of any but 'tadpole' forms. It is also to be remembered that in sheep, Case No. XXXIII, and goat, Case No. XL, the organism which appeared was of the 'tadpole' variety, whereas the guinea-pig from which they were inoculated had shown 'long' forms, and 'stumpy' ones were present at the time of inoculation. In none of our inoculated dogs were forms longer than 16μ seen. Most were of the 'tadpole' variety, but forms corresponding to 'stumpy' were encountered towards the end of the disease. We reserve the description of the organisms encountered in the naturally infected animals till later.

In rabbits, in the films made from our single case, only 'tadpole' forms were seen.

Guinea-pigs are the most satisfactory animals for revealing the dimorphic variations of this trypanosome. It has been noted that the average period of duration of organisms was ten days. During the first four days, 'tadpole' forms are almost exclusively present, giving place between the fifth and eighth days to 'stumpy' and 'long'; whilst at death, and on the two or three days previous, 'stumpy' forms predominate.

The 'tadpole' and 'stumpy' forms correspond to the descriptions given, while that of the 'long' form is based on its appearance in these animals. We do not hesitate to say that in many of these, particularly the so-called male forms, a flagellum whose length sometimes exceeded 10μ was seen free from cytoplasm. These forms

were present, though less numerous than the 'stumpy,' in blood inoculated into the goat, Case No. XXXIII, sheep XL, and rat LXXI, in which the 'tadpole' forms were reproduced.

Rats were infected by blood containing all forms. 'Tadpole' forms predominated throughout the first few days of the disease, while the 'stumpy' became more numerous towards death. 'Long' forms were very rare, but were seen in those inoculated from an ox, a goat, and a guinea-pig; but they were not seen after inoculation from a dog or a second goat infection. It is to be remarked, however, that a thorough examination of all slides has not been possible.

We have here, then, a trypanosome whose prevailing type in naturally and experimentally infected animals is short, measuring only 9·75 to 15·3μ, and from which there is a relative absence of a flagellum. This type under the influence of a different host, or under natural conditions in the same animal, assumes a distinct form which measures from 25 to 31μ in length, and possesses a flagellum which may be upwards of 10μ long. With the knowledge at present available there can be no hesitation in ascribing the name *Trypanosoma dimorphon*, Dutton and Todd, 1904, to such a dimorphic organism.

II. *T. VIVAX*, Ziemann

We have already remarked that the movement of this organism in fresh cover-glass preparation is of extraordinary rapidity, and the effect produced by the passage across any one field closely resembles that of a spirochaete, the corpuscular displacement being transitory and of no greater magnitude than that produced by drawing a floating hair across the surface of still water. It is impossible to retain any one organism in the field by movement of the slide, even when the mechanical stage is not employed, the parasite crossing and altering its direction with bewildering swiftness. In a preparation which has been kept for an hour or two the movements of some become more sluggish, and in fields where corpuscles are scanty, progression is seen to be due to a rotatory motion of the whole body, to the exclusion of a wavy undulating membrane and the vibrations or lashings of a free flagellum.

When one of these parasites is obstructed by corpuscles or fibrin threads, the action again differs from that of other trypanosomes. Sometimes the ordinary lashings are produced, but more frequently the posterior end becoming a fixed point, the anterior circles round with regular sweeps, forcing the corpuscles away as the arc-like radiations pass from a burning catherine-wheel. The trypanosomes measure from 20 to 26μ in length, and up to 3·4μ at the widest part, posterior to the nucleus. The body tapers anteriorly from this in a rather regular fashion, while the posterior end is distinctly rounded. The blepharoplast is large, round or oval, measuring up to 1·μ in diameter, and is usually terminal. The nucleus is commonly an elongate oval, up to 3·75μ in length and 2·5μ in breadth (average 3·4 by 2), and occupies nearly the whole transverse diameter at this level. The undulating membrane is very narrow, about 1μ, the bordering rim arises from the neighbourhood of the blepharoplast and is continued as the flagellum after running parallel to the body, so giving an aspect of stiffness to the whole structure. For the greater part of its length this 'free' flagellum is accompanied by a continuation of the periplast, which may or may not contain cytoplasm. The actual free extremity does not appear more than 3·5μ in length, commonly less; whilst the total length of the tapering end anterior to the cessation of the membrane is upwards of 8·5μ. The length of this whip was somewhat greater in the later stages of the disease, thus our figures in the inoculated calf, case IX, on August 4th (twenty-fourth day after inoculation) give an average of 3·4μ; those on September 8th (fifty-eighth day) vary between 3·4 and 8·5, with an average of 5·9μ.

The cytoplasm stains homogeneously; vacuoles are seldom seen and granules are not very common. Dividing forms are rare; in the peripheral blood we have only seen those with a double blepharoplast. We have never noted the alveolar arrangement of the cytoplasm described and figured by Lühe.[5] This trypanosome has retained its quite characteristic movement in fresh preparations and the same appearance in stained films, excepting only the slight differences in the length of the anterior extremity, in all animals which took the experimental infection.

III. THE TRYPANOSOMES OF NATURALLY-INFECTED DOGS

The forms seen in all three animals are the same. The trypanosomes measure from 20·25 to 28·9μ in length, and from 1·5 to 2·5μ in width. The protoplasm stains pink with Giemsa, vacuoles and granules were inconstant; the posterior extremity is most commonly pointed. The nucleus is situated towards the anterior part of the body, and varies in shape from a round to an elongate oval, the former taking a deeper stain than the latter. The blepharoplast is rounded, usually terminal, but may be removed from the posterior extremity by as much as 2·5μ. A well-developed undulating membrane is present, and the rim is continued as a flagellum, which varies from 3 to 10μ in length, and is accompanied, in the short forms at least, by a prolongation of the cytoplasm. Those carrying a short flagellum correspond in type to the so-called 'female' forms, and the long flagellar forms to the 'male' of other trypanosomes. It would be impossible to assert that these 'females' are not the 'stumpy' forms of *T. dimorphon*, which are somewhat larger than normal. Trypanosomes which could in any way be considered as having any relation to 'tadpoles' were not encountered; the smallest measurement made was 20·25μ. In the two guinea-pigs, the trypanosomes which reappeared measured from 21 to 30μ; in the smaller forms the projecting flagellum is short. The variations in length noted depend mainly upon the size of this flagellum; the body itself being fairly constant at 18 to 20μ. Tadpole-like forms were not seen at any stage of the infection.

In rats no forms except 'long' ones were met with. These measured from 21 to 30μ, and from 1·5 to 2μ in width. There is a distinct flagellar appendage in all, which differ from those seen in guinea-pigs only in being more granular and staining less deeply.

The natural dog trypanosomes are monomorphic in so far as no variations comparable to those existing between 'tadpole' and 'long' forms were seen. Being monomorphic and of large size, a parasite of a domestic animal and occurring in Africa, this trypanosome must be placed in that heterogeneous collection whose type is *T. brucei*.

ARGUMENT

It must be admitted that our present knowledge of trypanosomes does not permit of any satisfactory classification. In Africa the confusion in nomenclature is appalling, and the number of specific or suggested names, based largely upon the country of origin or the first found host rather than upon morphological or biological characteristics, renders absolute diagnosis of an individual form almost impossible without a typical living strain for comparison.

There is perhaps no great difficulty in asserting that the body structure of certain trypanosomes shows dimorphic variations under certain conditions. In all trypanosomes morphological differences between individuals occur to a greater or lesser extent, but only in the one species, *T. dimorphon*, Dutton and Todd, are they considerable. In this, unless the 'tadpole' and the 'long' be seen, they are not striking; and between the larger of the 'stumpy' and the shorter of the 'long' we do not consider the variations greater than between some of the so-called 'male' and 'female,' or the smaller or the larger forms of *T. evansi*. An additional difficulty is that there is no one structural point that can be seized upon as a basis for classification; most gradations can be met with in the shape of the posterior extremity and the length of the anterior and the amount of the flagellum that is free. As we have found forms recognisable as 'tadpoles' and 'long' both under natural conditions and those of ordinary animal experimentation, we have no hesitation in asserting that the cattle trypanosome first described is dimorphic according to the interpretation placed upon that word by workers on trypanosomiasis.

The first dimorphic trypanosome was that described in the Gambia by Dutton and Todd,[1] who at the same time described the pathological reactions produced by it there, which reactions were later confirmed in Europe by Thomas and Breinl,[2] and by Laveran and Mesnil.[3] They showed that rats, guinea-pigs and, with one exception, dogs are susceptible, and die within one or two months. The trypanosome encountered in the cattle of the Congo Free State was also dimorphic in type, but the animal reactions in that country differ from those obtained in the Gambia. At one post, Romee, laboratory animals were shown to be highly susceptible; at the other

posts twenty-nine inoculations were made from cattle whose blood showed trypanosomes of this type. One rat showed organisms twice, after the original and after a re-inoculation, but for one day only in each case; a second rat only became infected after several months' incubation. Dutton, Todd and Kinghorn [4] consider this Congo trypanosome as *T. dimorphon* on the grounds that the morphological characteristics of this species are peculiar in the genus *Trypanosoma*, and identical with those of the form they describe; and that the variation in virulence is not sufficient proof that more than one species of trypanosomes was present. The trypanosome which we describe shows animal reactions approaching those of the Gambian strain; all our dogs, rats and guinea-pigs have succumbed to an acute infection within two months, and, as in Gambia, the rabbit has a more chronic disease and the cattle an acute form. With similar morphological appearances and similar animal reactions, we consider the Rhodesian form to be *T. dimorphon*, Dutton and Todd, 1904.

We have much more hesitation in ascribing the second of the Rhodesian cattle trypanosomes to a specific class. The morphological characteristics seen in animals which were susceptible are not sufficient to consider it dimorphic; 'tadpole' forms, or any approach to these, were not encountered; the variations seen were not greater than have been found in monomorphic trypanosomes, and to this group it is assigned. We distinguish it from *T. dimorphon* which occurred in cattle of the same herds and also in some of the same animals by:—

1. The morphological appearances; the fairly constant size and the extreme rapidity.
2. The clinical type of disease induced; the great daily variation of temperature as opposed to the 'curve' in cattle suffering from *T. dimorphon*, and the absence of the striking febrile reactions seen in sheep and goats.
3. The animal reactions: Five rats, five guinea-pigs and three dogs were inoculated without effect. One goat and three sheep did not become infected, and in those which did there were indications of recovery. In one goat and one sheep which had been inoculated with this trypanosome, and were not showing parasites, a re-inoculation with

T. dimorphon brought about a febrile reaction and the appearance of this organism in the blood.

4. The *post-mortem* appearances: Splenic enlargement was not noted in the three cases upon which autopsies were possible, whereas in *T. dimorphon* infection it was a common feature.

Sander and Hennig [6] state that according to Ziemann *T. vivax* occurs spontaneously in cattle, sheep and goats; the incubation period is 5-8 days; and on *post-mortem*, enlargement of the liver or spleen is seen. Experimentally, grey rats died in 8-11 days; in donkeys the disease is chronic, and in a German dog the reaction was apparently doubtful. A negative result was obtained in a white rat. In Rhodesia white rats and native dogs were negative; the one donkey inoculated did not show organisms, but it has not been examined or reported on for three months and we are ignorant of its present state. A point of difference we note is the enlargement of the spleen, but our observations are based on three *post-mortems* only.

The morphology, as given by Lühe, differs from that of the form under discussion in possessing an alveolar protoplasm and a somewhat pointed end. These are minor points, negligible so far as the morphology of other trypanosomes is concerned, in which similar variations are commonly seen. The experimental work on both sides is limited, but the animals used are similar, and if Ziemann's dog was negative, and such is a possible interpretation, the results coincide, though we were unable to use grey rats and pigs in which Ziemann obtained positive reactions.

We know of no other trypanosome whose activity approaches that of this form. On morphological grounds, then, and animal reactions this trypanosome coincides more closely to that of Ziemann than to any other, and with all reserve, we feel justified in considering the second parasite of Rhodesian cattle as sufficiently closely allied to *T. vivax*, Ziemann, to bear that name until the classification of the genus be put on a more satisfactory basis.

TRANSMISSION OF CATTLE TRYPANOSOMES

European and native unite in incriminating the tsetse-fly common throughout the Northern part of the area of North-Western Rhodesia. The examinations of our specimens of these has so far only shown *Glossina morsitans*, the approximate distribution of which is marked on the attached map; but it must be understood that the lack of signs in certain areas does not imply freedom from the fly, but only that we have no positive knowledge of its occurrence.

Tabanidae have only been incriminated in the one instance already recorded. During June, July and August, 1907, we did not see any, but during the latter end of September they were occasionally seen at our camp, and they were very numerous in Broken Hill on the first few days of October. On our line of march they were constantly encountered, and may be held as having an almost universal distribution. The larger members of this family are locally known as 'hippo flies'; *Haematapota*, also common in November and December, are usually spoken of as 'blind flies.'

Stomoxys were taken in the cattle kraal of the farm where our camp was established in July and August, and they were caught on the River Kafue in November and on the River Luapula in December. They were most frequently met with in villages, but on two occasions were taken from recently shot game.

Lyperosia were caught in the same cattle kraal in July and the first week in August, and again during the latter part of September. They were not seen in the interval, nor have they been taken anywhere on the route followed.

Hippoboscidae are, in comparison with India, rare. One specimen of *H. rufipes* (?) was shown us as coming from near the Kafue. We have thrice taken *Lipoptena* on dead buck.

Owing to the relative frequency of *Glossina* and their association with game and domestic animals, there can be no question that, assuming game to be the pre-existing reservoir, they are the most capable flies for transmitting the trypanosome to cattle or other domestic animals taken into their haunts. We may mention that we have seen trypanosomes in fresh preparations of blood made from a recently shot Hartebeest (*Bubalis lichstensteini*) and a bush buck (*Tragelaphus scriptus*), but our examination of the slides and inoculated animals is incomplete.

Nearly all the cases of trypanosomiasis in cattle examined could be given a history, often very imperfect, of having at some recent date been exposed to the tsetse; but in one herd, where the history is reliable, the evidence is suggestive that *Stomoxys* and *Lyperosia* had acted as transmitting agents.

Forty-four animals from this herd had lived at Kapopo, which itself is free from fly, for upwards of three years, during which time they had always been in good health and deaths were rare. In August, 1906, they were brought to Broken Hill, a distance of about 97 miles, following as far as possible a route where tsetse were scanty or not known to exist, and adopting the usual precautions, such as marching at night. They remained in perfect health until June, 1907, when they came under observation. During these eleven months two animals had died, apparently of some acute inflammatory disease, and one had been destroyed as the result of an accident, and six others which had not been recently in tsetse areas were added. The farm where these cattle were kept is at least two and a half miles from the nearest known fly area, and they were all employed on this farm in agricultural work; the three cows and three bulls grazing close to the buildings. On April 18th, six bullocks were sent on a journey of thirty miles Southwards to Mwomboshi, and returned four days later. *G. morsitans* occurs on the road travelled about eight miles from the farm, but it is limited to a narrow patch. On June 25th, three of these animals and one which had not been away showed trypanosomes, and they were all dead within a month; one died within ten days, and this at the height of the dry season, when deaths are said not to take place. Four other animals were suspected, but did not then show organisms. The rest of the herd were all in good condition, looking bright and doing the hard agricultural work well. On July 18th, all remaining animals were examined, and eleven, some of them straight from the plough, showed trypanosomes. The owner, who possessed a small microscope, picked out another on the 22nd, one on the 27th, and we found two more on the 29th. Three of these were cows which had not been exposed to *Glossina* for twelve months at least.

All these animals were segregated, and those not showing organisms were placed by themselves in a kraal and grazing area which appeared free of all biting flies, such as *Stomoxys* and

Lyperosia. Further examination of these apparently healthy animals weeded out five more cases in August. The balance, fourteen, continued healthy, and of these thirteen were taken for transport work in September. They did the work well, and showed no greater death-rate on the 400-mile march than did the other ninety cattle taken.

After the first examination, at which only suspected cattle were presented, all animals inspected were, without exception, free of any clinical signs of the disease. These became manifest in about two weeks time, and death took place within an average of thirty days of diagnosis. This is the average between these dates in fourteen apparently healthy cattle, and excludes all those upon which experimental work on treatment could be held as influencing the course of the disease in either direction.

With a disease of such rapidity and virulence, we consider it highly improbable for the infection to have lain dormant in the animals since August, 1906. From an examination of all conditions, we think it probable that one or more of the six cattle which went to Mwomboshi in April contracted the disease on the road and brought it to the farm, where, in the presence of *Stomoxys* and *Lyperosia* in the kraals, these animals, including cows and bulls, which did not leave the place, became infected, and that the segregation from these flies checked its spread to the fourteen cattle which remained healthy. The one animal which did not go on trek with the thirteen cited above was brought to live with the sick on September 10th. It was then in excellent condition, but on a visit to the farm on November 20th it showed *T. dimorphon*, and would in all probability succumb within two weeks. Owing to the lack of facilities, and the pressure of other work, we were unable to conduct any transmission experiments with these two flies, but as affording corroborative evidence we examined the road on which the outbreak was suspected of having originated. We were informed by the owners concerned that they had lost animals during the present year after travelling through this area with their spans, and this despite the usual precautions. We, therefore, exposed two healthy bullocks to *Glossina morsitans* there.

CASES No. XLIV, XLV.—Two animals from small herd at Mwomboshi, where they had lived for between two and three years, and where stock apparently does well. August 19th, 1907. These two were driven between 10 a.m. and 1 p.m. over the road, through the area inhabited by *Gl. morsitans*. Only three flies were

seen to feed, two on No. XLV, and one on No. XLIV. On arrival at our camp they were segregated and carefully kept from all association with other cattle. The temperature remained normal until September 3rd, the fifteenth day after being bitten. On September 6th, the eighteenth day, trypanosomes were seen in the blood of both. This trypanosome was morphologically identical with that we have called *T. vivax*. On September 13th (XLV) and September 16th (XLIV) *T. dimorphon* appeared, and both organisms were present until September 30th, when No. XLV died. Both had been submitted to treatment by Atoxyl and Mercury, and No. XLIV has been detailed elsewhere. (vide chart ii.)

This double infection by such a small number of flies as were seen, and on a road where game is scarce, caused further enquiries to be made, and we found that a span of oxen had travelled that road on August 14th, five days prior to the date our animals passed. On September 21st we examined this span, and found two animals infected with *T. dimorphon*, and a third showing both *T. dimorphon* and *T. vivax*.

If the *Glossina morsitans* which bit Cases Nos. XLIV and XLV had not derived these organisms from game, it would appear that they have the power of transmitting five days after the infecting feed, which would have been taken from this span, whose owner reported that he had lost several animals during the months of June to September from what he regarded as 'fly' infection.

CONCLUSIONS

1. That trypanosomiasis of domestic stock is very prevalent in the Northern area of North-Western Rhodesia, and that it is due to *T. dimorphon* (Dutton and Todd), *T. vivax* (Ziemann), and one morphologically allied to *T. brucei* (Plimmer and Bradford). *T. theileri* also occurs, but does not appear to cause serious damage.

2. That these trypanosomes may be transmitted by *Glossina morsitans*, *Stomoxys calcitrans*, and a species of *Lyperosia*. In nature it will depend upon the conditions under which cattle are maintained, to which of these genera special attention must be paid in prophylaxis.

3. That treatment, as detailed in a previous report, shows certain indications of success, and we urge facilities for a continuation of the work on this disease, which tends to stagnate the proper development of a wealthy mineral and agricultural country.

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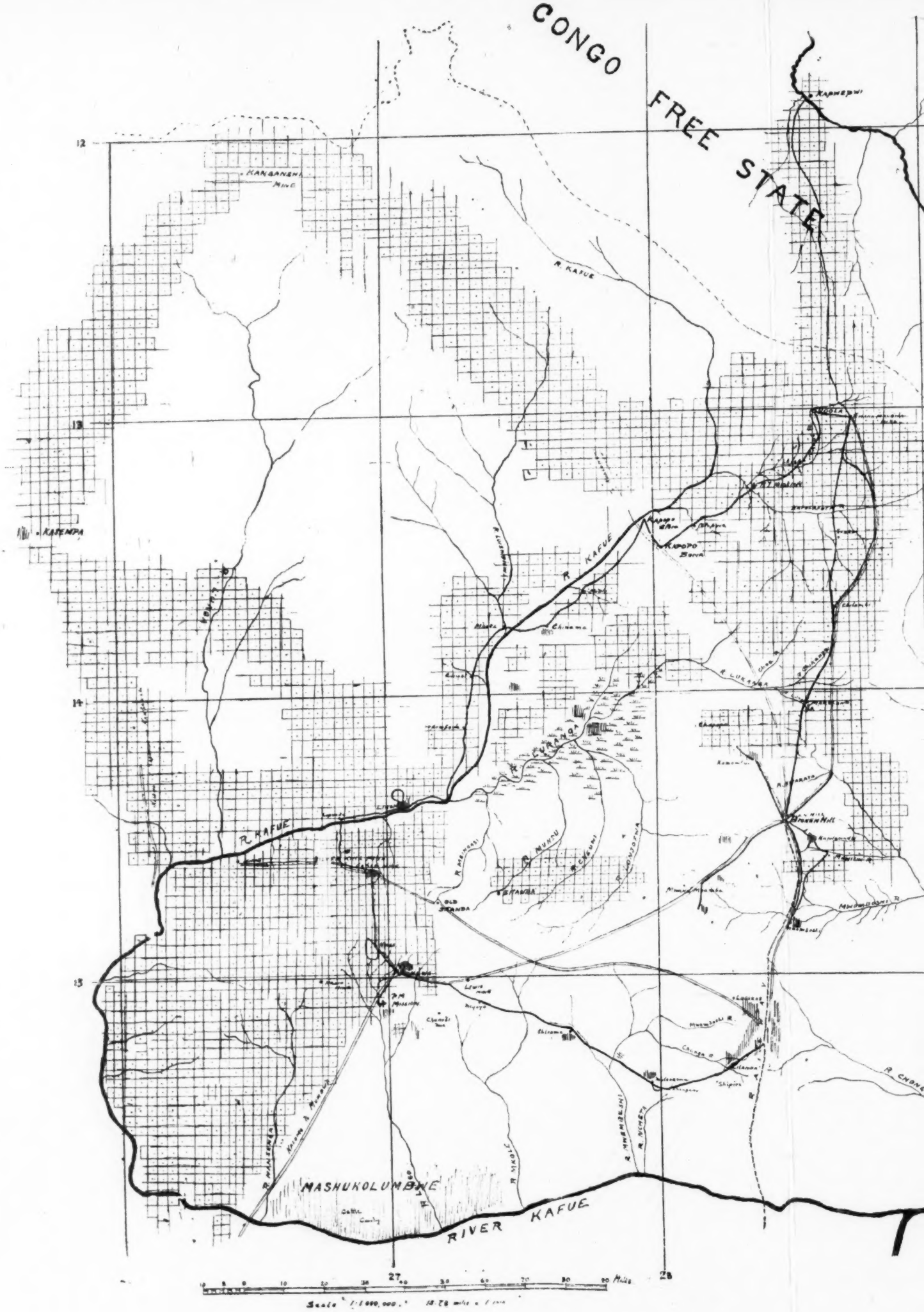
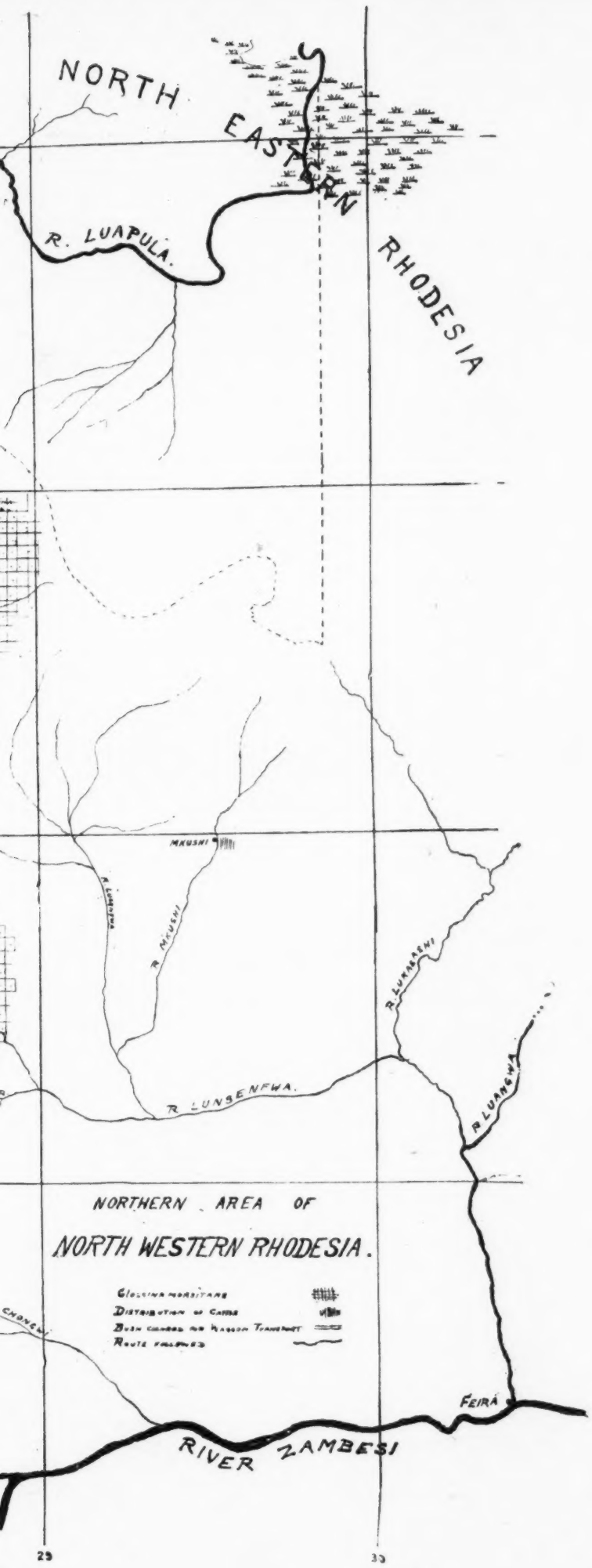

CONGO FREE STATE
Kapwepwi
12
13
14
15
27
28
KANSANSHI Mine
R. KAFUE
KASEMPA
Kapopo Bona
Mkota
Chinama
R. KAFUE
R. LURANGA
R. LUKANGA
Broken Hill
Kapiamondo
Mwomboshi R.
Chombi
R. MUNYU
R. CHIGUNI
R. LUNSEMFWA
OLD SHANDA
SHANDA
P.M. Mission
Lewis mine
Chirama
Mwomboshi R.
Chunga R.
Shipiro
R. CHONGWE
R. MWEMBESHI
R. NCHETA
R. MKOLE
R. NANSENGA
R. LUMBO
MASHUKOLUMBWE
Cattle Country
RIVER KAFUE
10 5 0 10 20 30 40 50 60 70 80 90 Miles
Scale 1:1 000,000. 15.78 miles = 1 inch


NORTH
EASTERN
RHODESIA
R. LUAPULA.
MKUSHI
R. MKUSHI
R. LUKASASHI
R. LUANGWA
R. LUNSENFWA.
NORTHERN AREA OF
NORTH WESTERN RHODESIA.
FEIRA
RIVER ZAMBESI
25
30

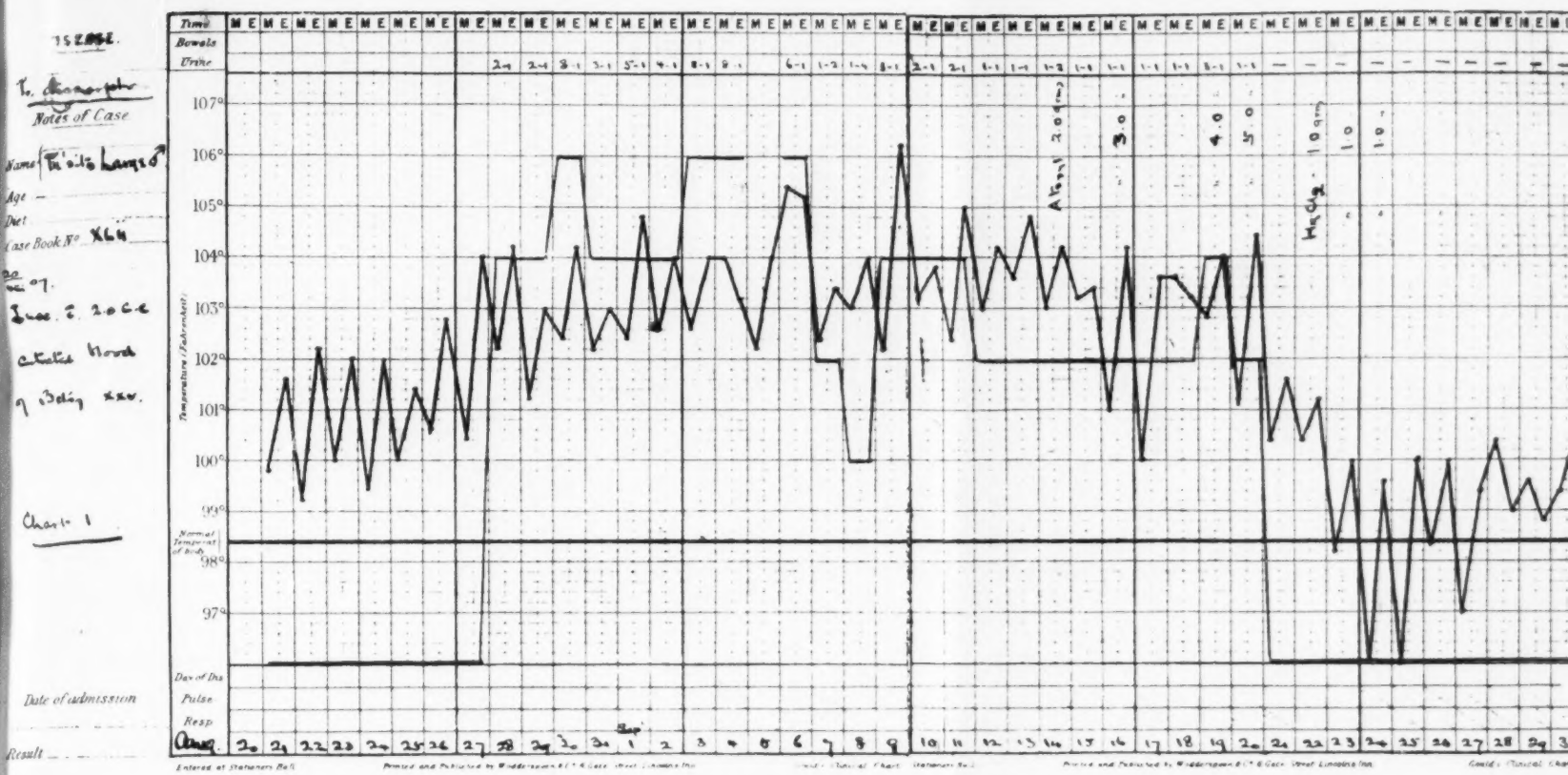
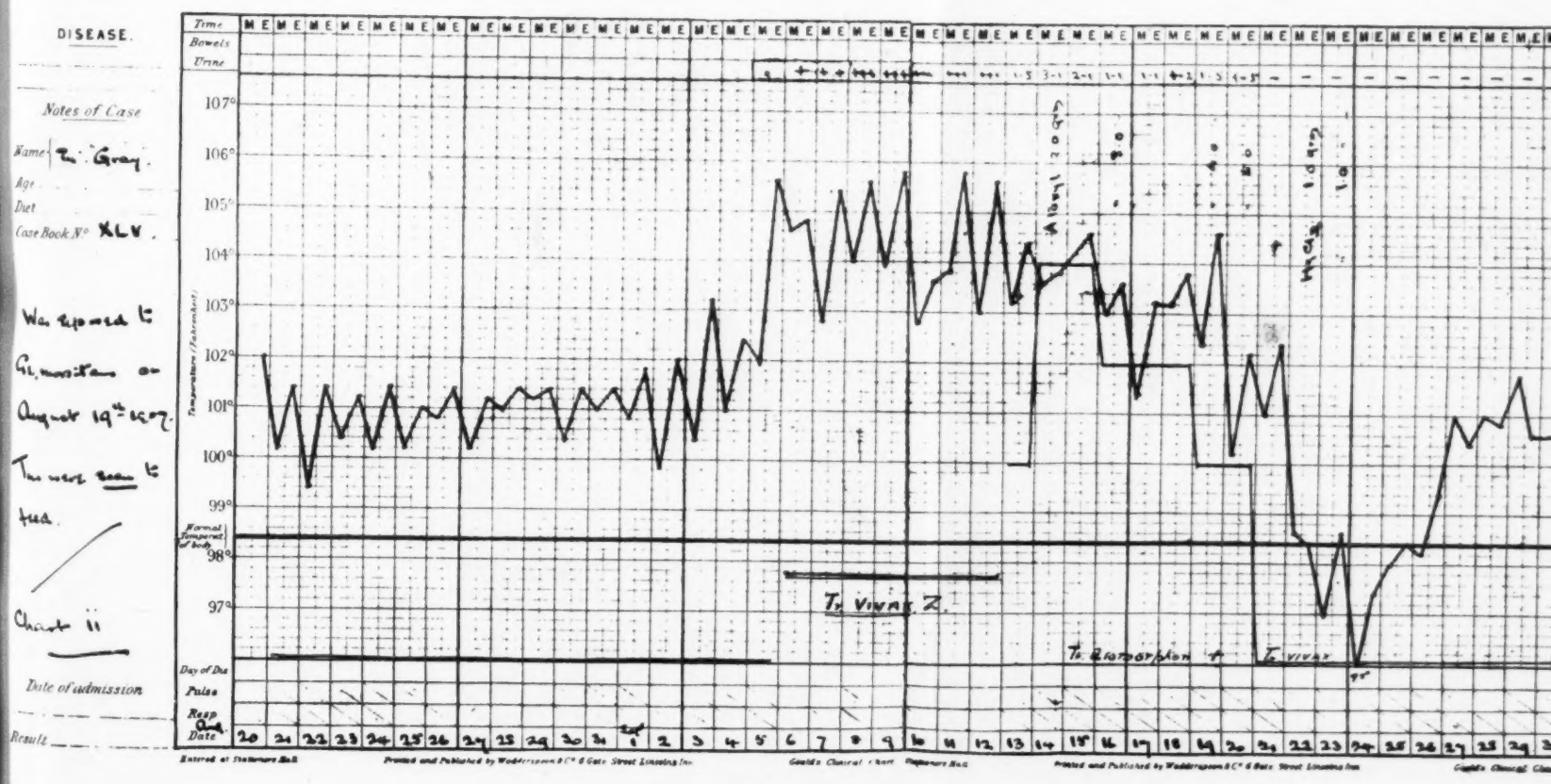


CHART I



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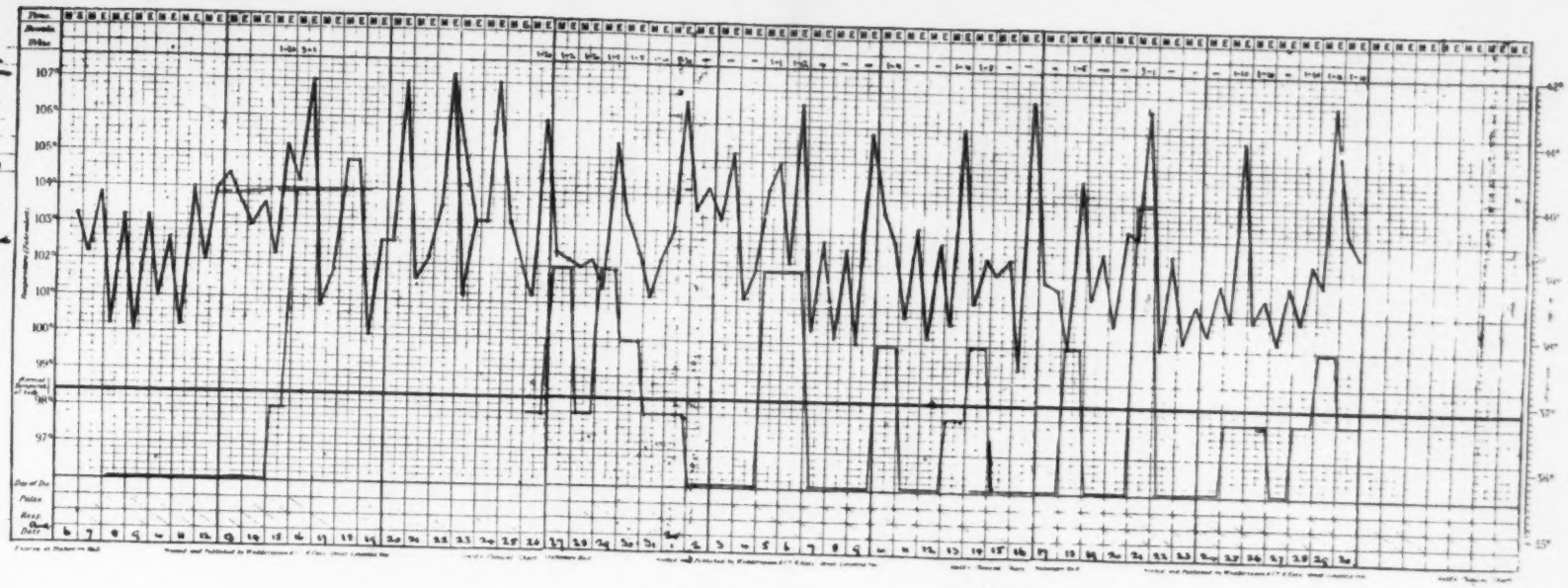

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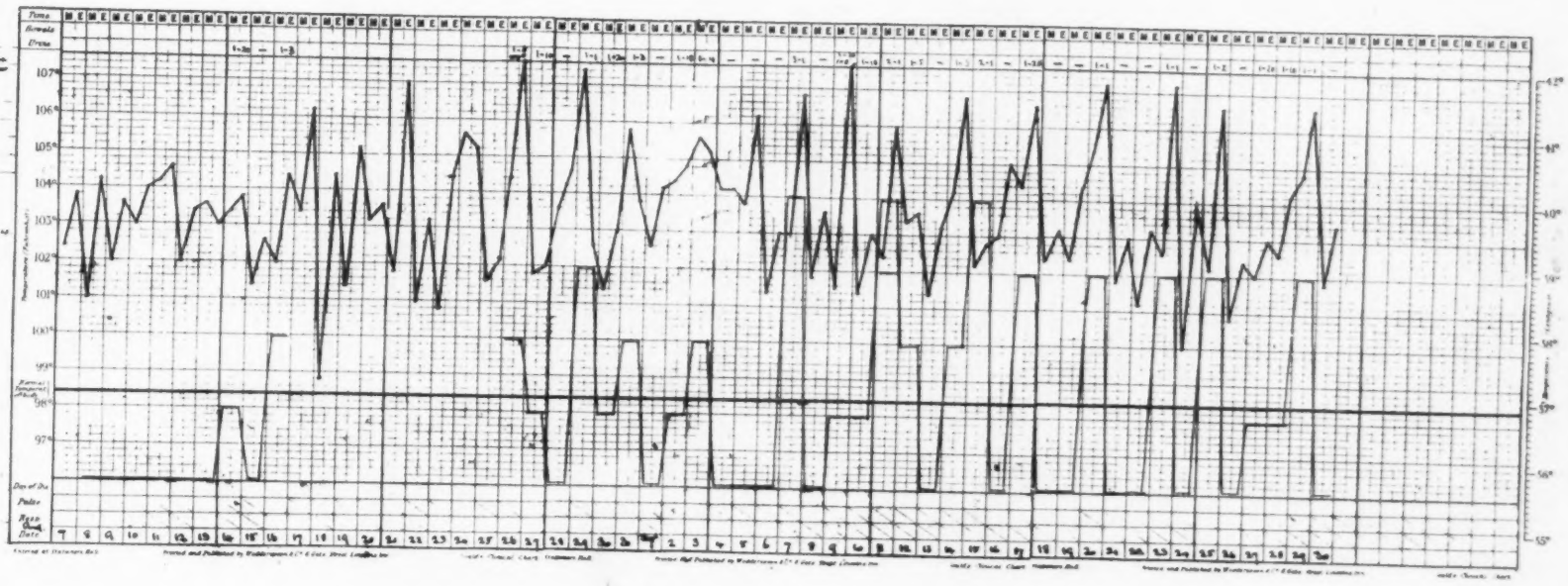

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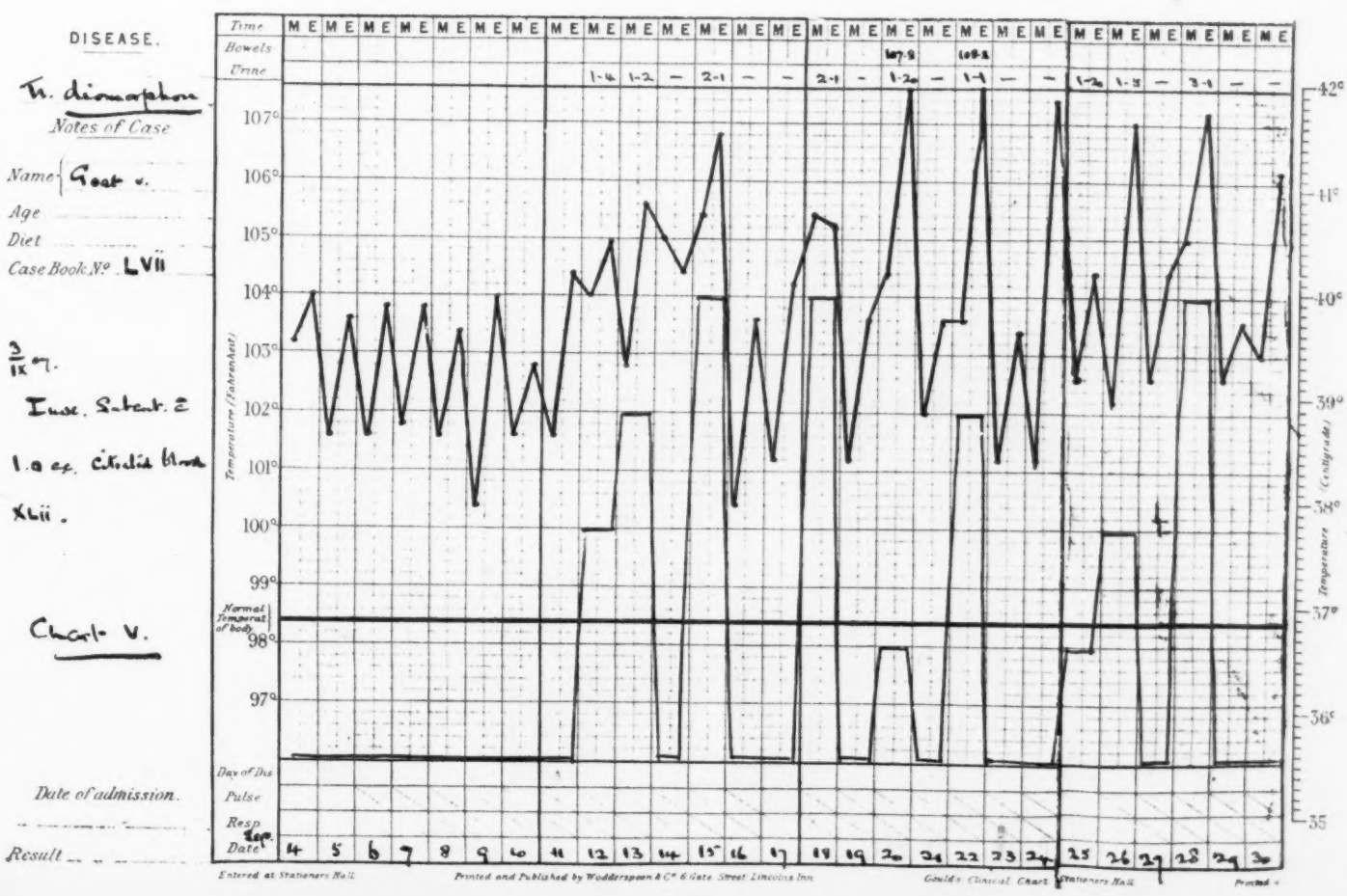

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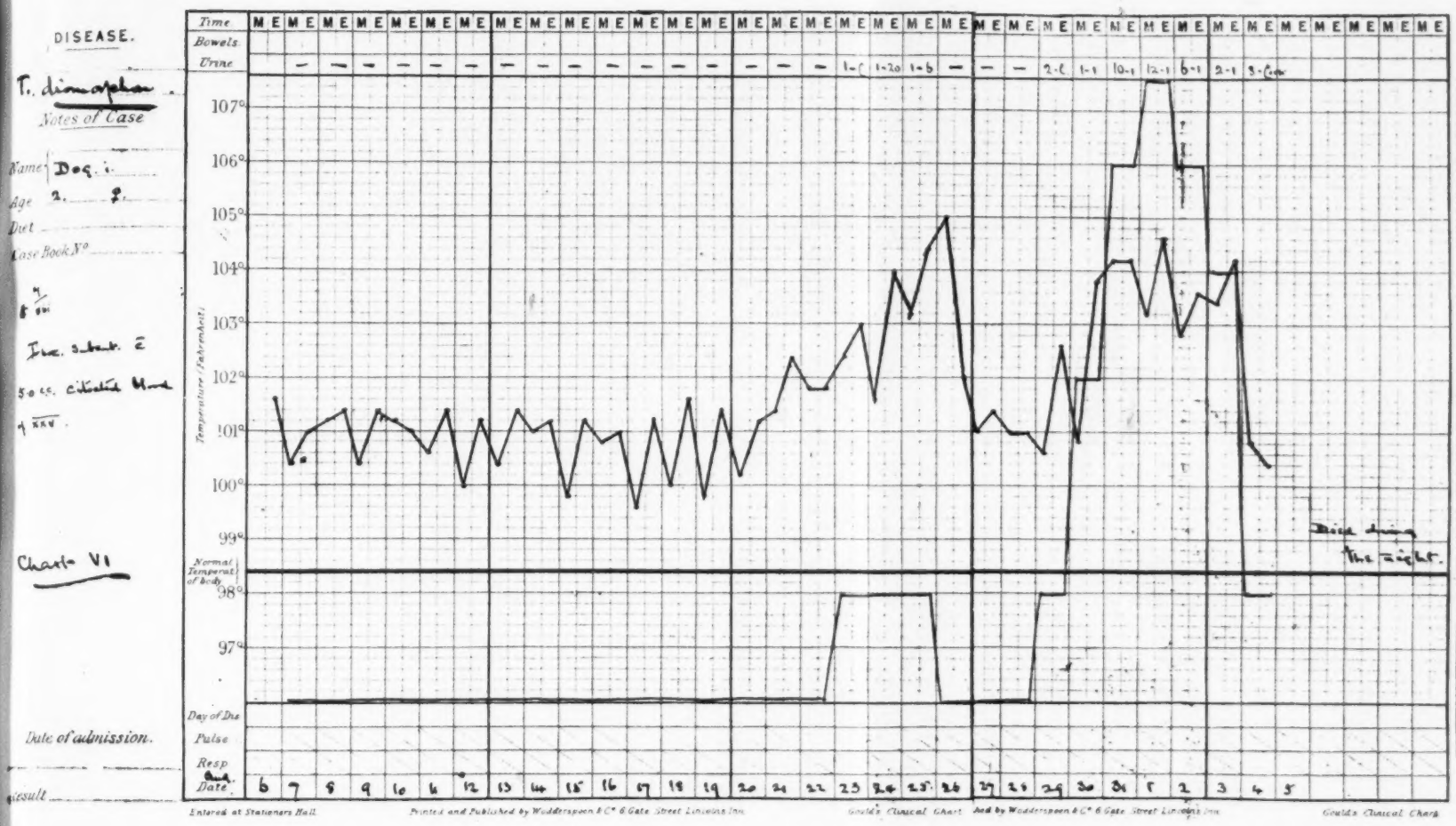

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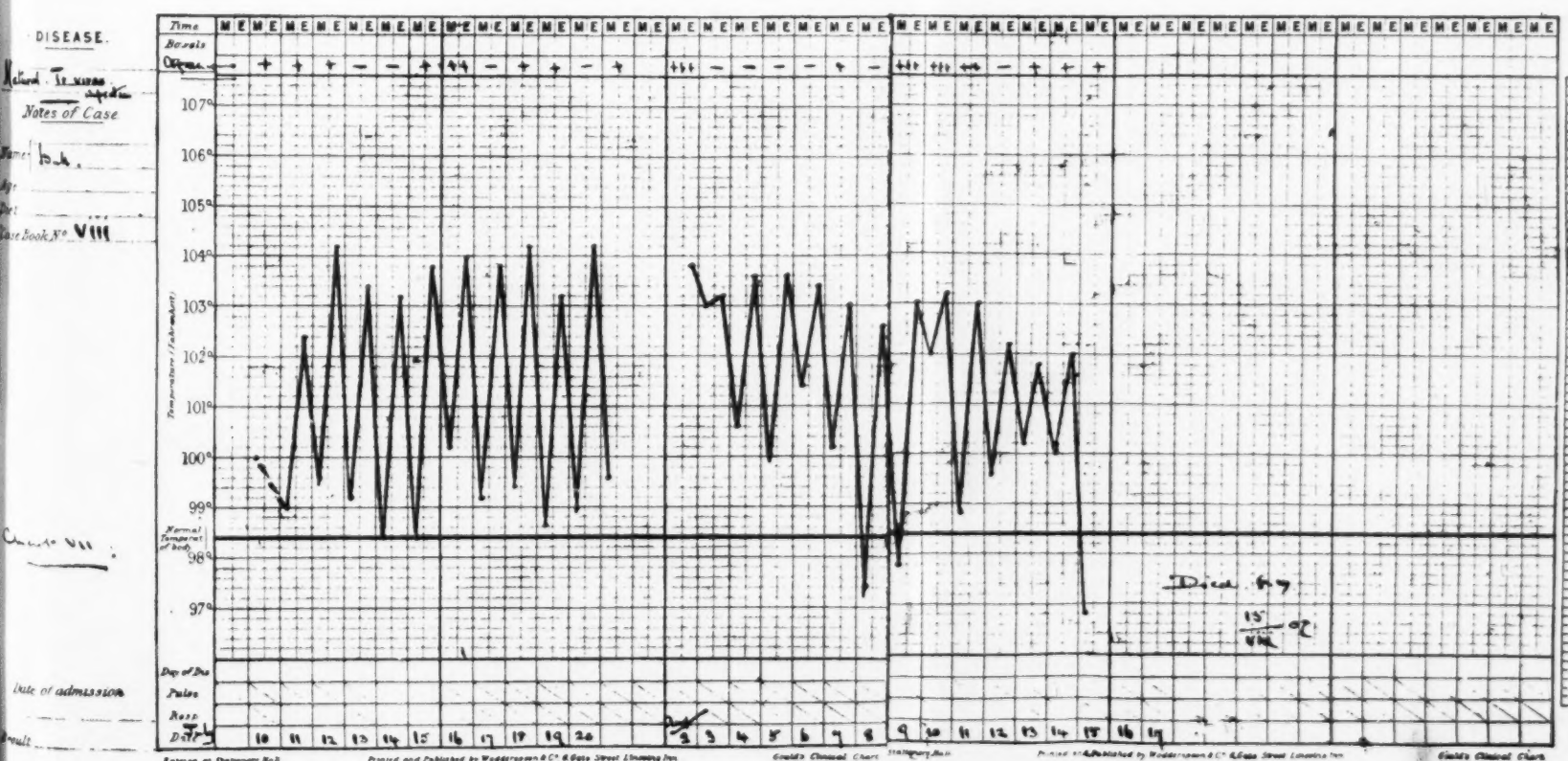

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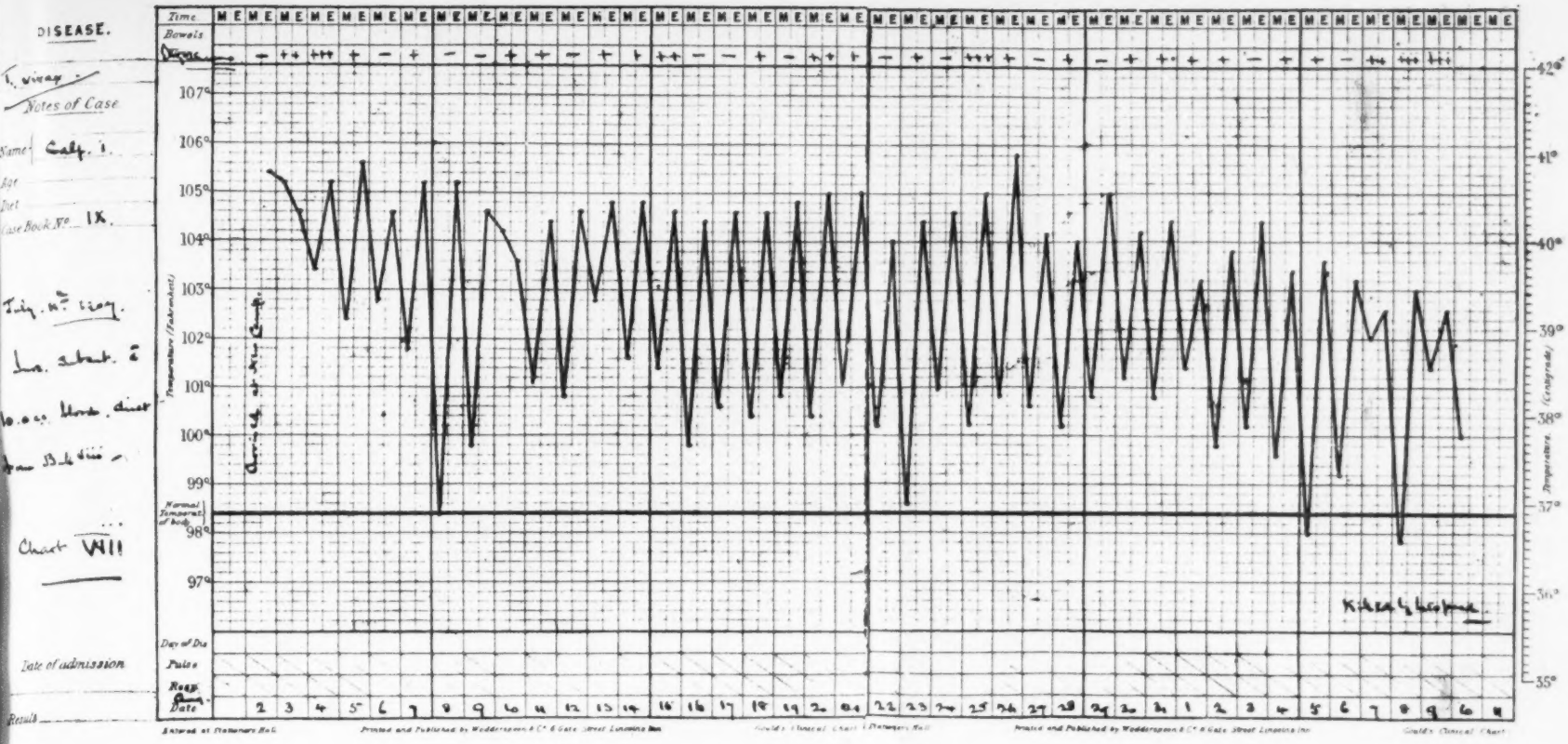

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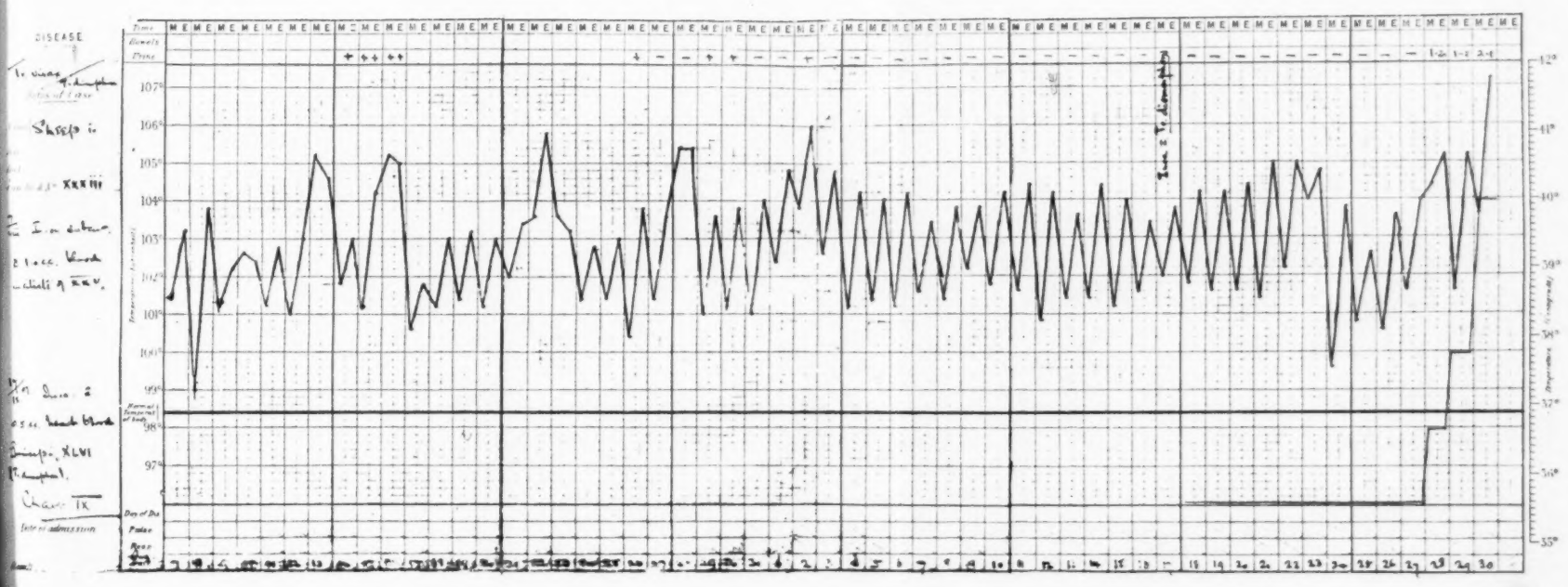

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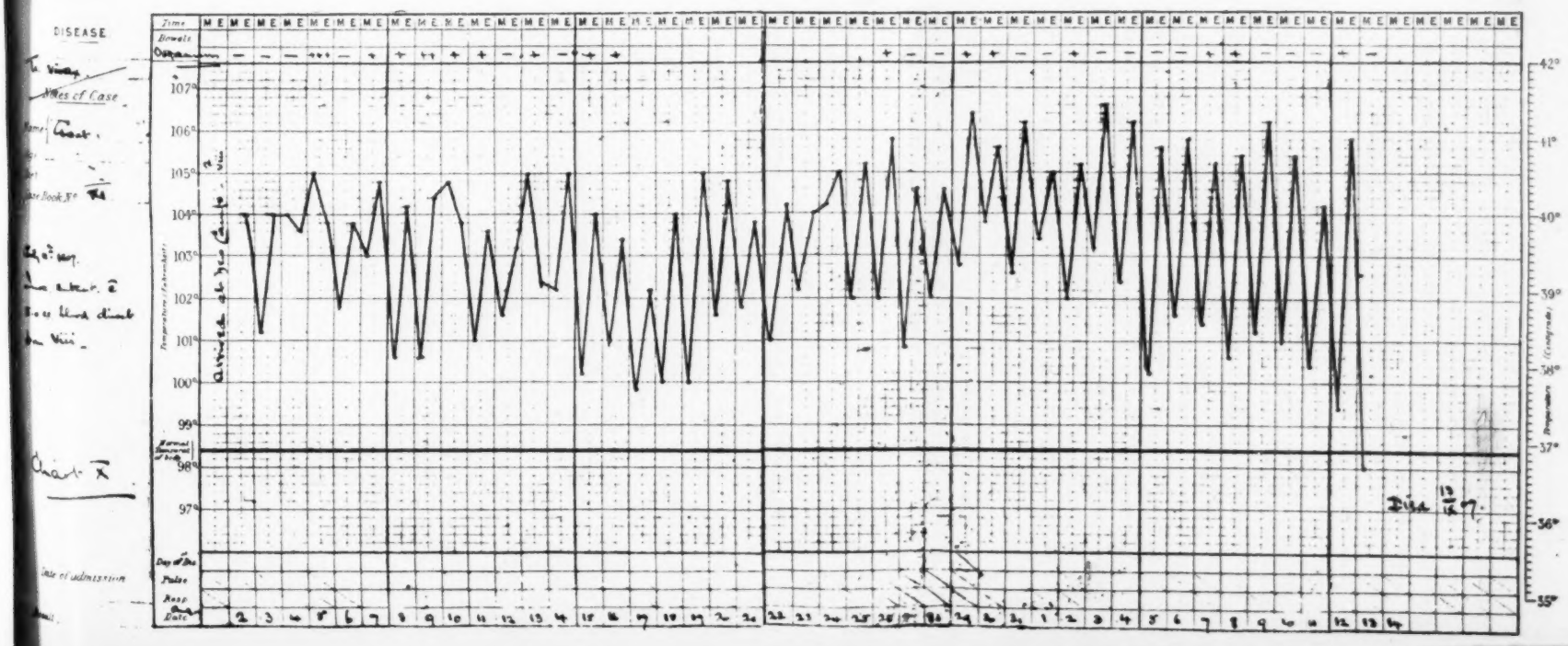

REPORT ON THE WORK OF THE GREEK ANTIMALARIA LEAGUE DURING THE YEAR 1907

BY

M. HADJIMICHALIS, PRESIDENT

AND

JEAN P. CARDAMATIS, GENERAL SECRETARY

(*Received for publication* 13 *April*, 1908)

I

Greece takes rank among those countries which are the most infested by malaria.

This plague existed here even in the remotest periods of antiquity, and Hippocrates, the father of medicine, not only mentions in his works all the various forms of the disease, but he also was aware of its connection with marshes, and of the influence of certain meteorological conditions, especially the frequency of rain, upon its development.

We know that malaria has always existed in Greece since that time, and Professor Ross is certainly correct in attributing to a great extent the cause of her misfortunes, to this dreadful scourge.

Official statistical information, concerning this disease, exists only for the last nine years, and is limited to the 12 largest towns of Greece, with a population of over 10,000 inhabitants each, or a total of 446,743 souls.

According to these statistics (Table A) it is shewn that the average annual number of deaths from malaria, in the 12 towns referred to, is 287 or 9·8 per 10,000 inhabitants.

The first place, as regards the number of deaths, is held by Volo, in Thessaly (21·89 per 10,000); next in order follow Pyrgos, in the Peloponnesus (19·48), Larissa (17) and Triccala, in Thessaly, (14·72) and Calamata, in the Peloponnesus (13·38).

It must, however, be observed, that these figures do not give an exact idea of the prevalence of malaria throughout the country, as

this disease is much less frequent in the towns than in the rural districts.

That this is the case is clearly shewn by the information which has been collected by the League, and in the publications of many physicians. In fact malaria is so widely spread in our country that scarcely any communities are free from the disease.

The plains of Thessaly, Phthiotis, Acarnania, Boeotia, Elis, Messenia, Argos and Laconia are all severely scourged by this plague, and, at certain periods, hardly a single inhabitant of those districts escapes the disease.

For instance, an examination of the school children, held at Marathon in October, 1906, shewed that enlargement of the spleen was to be found in every pupil (100 per cent.).

The disease usually begins in the month of May, reaches its height in July and August, and commences disappearing in November, but in the more elevated districts it appears later and disappears earlier. However, even in winter relapses are very frequent.

Further statistical information may be found in a small work, published by Professor C. Savas (Le paludisme en Grèce et l'œuvre de la Ligue Antimalarienne. Atti della Società per gli Studi della malaria, tome VIII, p. 139, 1907).

II

The year 1907 was signalised by a severe epidemic of malaria in Greece, as was shewn by the private information which reached the League from all parts of the country, as well as by official statistics (Table A).

As regards, however, the number of cases of the disease, the year 1907 fell short of 1905, whilst the intervening year, 1906, was characterized by a considerable decline in this respect.

In 1907 the greatest number of deaths from malaria took place in July (Table B), August shewing the second highest results, but whilst in the previous years June and September shewed the highest figures, on the contrary, in the year under review a slight decrease in the mortality from the disease was observable in September, followed immediately by a fresh increase in October, which was maintained throughout November. This curious fact, which was observed for

the first time during this year, is to be attributed to the prevailing atmospheric conditions, as, after a warm summer, lasting until the middle of August, the temperature fell in September, after which it again commenced to rise and remained relatively high during October and November.

Consequently, the disease, which had commenced to abate in September, began to re-assert itself in October, and continued in this way throughout November, and even, to a certain extent, in December.

The records, as regards fatal cases of malaria in the year 1907, were held by the Thessalian towns, especially Volo (with 45 deaths per 10,000 inhabitants) followed by Triccala (24) and Larissa (23). The second rank was filled by two Peloponnesian towns; Pyrgos (15 deaths per 10,000), and Calamata (13).

The capital, Athens, itself was severely afflicted by this plague during the year under examination, for, whilst the average annual number of deaths from malaria in that city (as shewn in Table A) is 56, during 1907 no fewer than 71 persons died of the disease, or, in other words, that year was second only to the year 1901 in respect of the number of fatal cases of malaria.

The cause of this prevalence was found to be in the stagnant pools remaining in the river-bed of the Ilissus. The draining of the Ilissus was commenced some three years ago, and those quarters of the city, bordering on the drained portion of the river-bed, were found to have been but slightly affected by the disease this year, whilst the portion of the city adjoining the section of the river where the draining operations had not yet been completed, were severely attacked.

III

One of the chief cares of the Antimalarian League, after its establishment three years ago, was the collection of information concerning the propagation of malaria.

In view of the fact that the official health statistics are confined entirely to the publication of the number of deaths from various diseases in the 12 largest towns of the kingdom, the League endeavoured to supply this deficiency as far as possible, and with this object a printed circular was addressed to all the physicians with the

request that they would fill in their answers to the questions therein asked. This was also done in 1907. At the same time a bulky volume was issued, containing all the material accumulated by the League during the years 1905 and 1906 (together with the information gleaned by the Panhellenic Congress of 1901) consisting of the reports of 450 physicians on malaria in three hundred demes of the country.

It is hoped that the Statistics of the remaining 120 demes will soon reach us (as we are quite without information as to the progress of the disease in these demes), and the malaria chart of the Kingdom will thus be completed.

This volume in addition contains the transactions of the League during the two years 1905, 1906, together with a number of scientific articles, and was issued in an edition of 3,000 copies and distributed gratis to all the physicians of the Kingdom, so as to serve as an incentive to their aid in the common struggle against malaria.

We also sent 200 copies to the medical men of Crete, accompanied by a circular requesting information respecting the disease in their island during the year 1907.

The High Commissioner of Crete, Mr. A. Zaïmis, was pleased to evince the greatest interest in the suppression of malaria, which infests that island, and he gave the necessary instructions for the commencement of a methodical campaign against the disease.

The Councillor of the Interior also promised to do all in his power to assist in the campaign in view of the progress which has already been made in Greece.

Before proceeding to more important work, the League considered it better to obtain the advice of some of the more eminent provincial medical men, who are actually engaged in the field of battle, and who would be able, by their experience and valuable advice, to afford us considerable assistance.

The summoning of these physicians to Athens would be another considerable advantage to the League in its work, as they would be thus enabled to come into closer contact with us, even on a short visit only, and would be able to discuss the newest ideas, in connection with the propagation and suppression of malaria, and, on their return to their own districts, they would be able to act as apostles of the League and to endeavour to instil the principles of the League by

means of lectures, &c., and in other ways to further the aims of the League.

Inspired by these ideas, the Committee decided to invite to Athens, at the expense of the League, a number of these medical men, and, on the 3rd and 23rd of May O.S. two medical meetings were held and were attended by about 50 physicians from the different provinces of the Kingdom.

Each meeting lasted only two days, and the best means of combating the malarial disease was discussed. With the aid of the lantern and the microscope, in the Laboratories of the Hygienic and Pathologic Anatomical Institute, the malaria parasites and the various kinds of mosquitoes were shewn upon the screen, whilst the causes of malaria and the means of protection against the disease were explained at length in accordance with the most up-to-date theories. Visits were further paid to the bed of the Ilissus river and the breeding places of the Anophelines in the stagnant water.

We consider that the assembly of these doctors at Athens met with complete success, and that it will conduce to the dissemination of the work of the League, as they have already formed, in their own districts, centres for the inculcation of the ideas of the League among the other local medical men.

It gives us great pleasure to mention that only three of the doctors attending the Congress asked for, and received, their travelling and hotel expenses.

The President has received letters from some of these gentlemen, shewing that they are indeed making great efforts to realise the aims of the League, as they have both delivered lectures and have approached the local municipal Councils with proposals for the voting of funds, besides collecting money themselves for the purpose of draining the pools in the neighbourhood of the various communities.

The action of the Board was not limited to the convocation of the physicians, but, actuated by the desire to place the information concerning the means of combating the disease before as wide a circle as possible, so that the people might be furnished with the most up-to-date ideas, the League printed and distributed gratis 30,000 copies of a pamphlet containing information regarding the propagation of malaria and instruction as to the means of prevention.

In addition, the General Secretary of the League, Dr. J.

Cardamatis, was dispatched to 30 towns of the Kingdom in order to deliver lectures on the same subjects, which were attended by a large number of people, including the medical men and local authorities.

By means of these lectures, together with the distribution of printed instructions, and the indication of the anopheline mosquitoes, the interest of all was aroused with regard to this vital question.

The expenditure incurred in connection with the meetings and the lecturing tour of the General Secretary, amounted to Drs. 1.917.55 (about £70). The most important work undertaken by the League in the year 1907, was to combat malaria at Marathon.

The League considered that lectures and publications, and similar measures were not sufficient to achieve its eminently practical aims, but that practical application of the measures recommended by the League was also necessary for the persuasion of the public as to the efficacy of the remedies recommended, whilst, on the other hand, a precedent should be established for the execution of similar works in the future.

This advice had also been given by the Liverpool School of Tropical Medicine in its most valuable report on the suggested anti-malarial measures in Greece, dated the 25th of January, 1907.

The League, however, had other reasons for considering this work to be necessary, as we ourselves should study, in practice, the conditions under which the combating of malaria in Greece was possible, taking into consideration the social and local peculiarities of the country. With this object the Committee selected Marathon as the field of action of the League.

In this respect Marathon presents many advantages, as it is, in the first place, one of the districts which suffers most from malaria; secondly, the plain of Marathon is separated from the surrounding districts by a range of hills, so that it forms a completely independent region; and finally, it lies at a convenient distance from Athens, so that the work could be carried on under the perpetual surveyance of the League. The sole disadvantage connected with the choice of that district lay in the fact that the number of inhabitants is rather large for the purpose (1,680 souls) and is scattered throughout the plain, thus rendering the expenses rather heavier than would otherwise be the case.

The work was commenced on April the 27th, by the establishment

in the village of a permanent Ambulance consisting of one doctor with a medical student as assistant, whilst the General Secretary of the League remained there alternately with the assistant of the Bacteriological Laboratory at the University, so that the Staff always consisted of three members, under the general direction of Professor Savas and Dr. Cardamatis.

The plain of Marathon lies at a distance of 36 kilometres from Athens, and contains three villages, Marathon, the capital of the deme, with 1,200 inhabitants, Bey, with 150, and Souli, with 160 inhabitants. There are, in addition to these villages, several small hamlets scattered here and there in the plain, inhabited by 173 souls in all, giving a total population of the plain of 1,680.

According to the statements of the local doctors, the number of cases of malaria averages between 80 per cent. and 90 per cent. of the population. Dr. Papasotirios examined, during the month of October, 1906, the spleen of all the pupils of both the schools, and found them enlarged in the case of 100 per cent.

Besides this, of 1,216 individuals, whom we examined in the month of May O.S., 1,031 or 85 per cent. admitted that they had suffered from marsh fever during the previous summer. This large number of cases of malaria is chiefly due to the neighbouring river-bed, the waters of which decrease in volume in the summer months, and leave pools full of larvae of *Anopheles superpictus.*

The peasants are also inoculated with the disease during the night, when sleeping in their vineyards, which are situated in the plain, close to several pools and two large marshes, in which breeding-places of Anophelines were always discovered, and especially *Anopheles claviger*, *superpictus* and less often *bifurcatus.*

The work was commenced in two ways, first by ridding the waters of the river-bed of the Anophelines, and, secondly, by the regular distribution of quinine to all the inhabitants as a curative and preventive measure.

The waters of the river-bed were concentrated in a narrow channel, in order to assure a rapid flow, and the pools were covered with petroleum once a week.

This work was partly carried out by workmen, but chiefly by the pupils of the schools, who gladly assisted under the leadership of their teacher, and they were thus afforded an opportunity of a practical

lesson as to the manner of communication of the malaria, and the means of safeguarding themselves against the disease.

The second method of prevention consisted in the distribution of quinine. Owing to the difficulty of supervising the inhabitants as regards the use of quinine, consequent on the manner in which they are scattered throughout the plain, and to the insufficiency of the medical staff, we preferred giving out the quinine according to the Koch's system, which in practice was slightly modified, e.g., the drug was distributed on both Saturdays and Sundays, in doses of 1 gramme each day. We must, however, confess that we often met with considerable difficulty in the application of this mode of distribution, as the giving of a comparatively large quantity at once caused much inconvenience to the people, hindering them in their work, or rendering their Sunday's rest burdensome.

During the whole of the six months of our work, we distributed 23 kilogrammes of quinine and 1 kilogramme of euchinine. The euchinine and 5 kilogrammes of sulphate of quinine were presented to us by the Jobst-Zimmer firm, whilst the Italian Government was kind enough to send us 3.100 kilogrammes (3 1/10) of the State quinine, prepared in the manner in which it is sold by that Government in Italy.

The latter method of putting up quinine was very much appreciated by the peasants, and the children, especially, readily took the tannate of quinine with chocolate.

Our experience with the euchinine was not so favourable, as it proved to be much less readily taken than the tannate with chocolate, and less efficacious.

The quinine provided by ourselves was the sulphate, and was distributed in wafer covers.

Of the 1,680 inhabitants of Marathon 1,544 underwent the treatment, but we are without information as to the result of the cure as regards many of these people. Of 1,252 persons, however, we possess the necessary information, and of these only 597, or 47·6 per cent., were attacked by the disease.

A more detailed examination of these figures shews that of 67 persons who took quinine for 21 to 24 weeks, none were attacked by malaria. Of 145 who took the drug for 16 to 20 weeks, 36 suffered, or 20·6 per cent. Of 220 who took quinine during 11 to 16 weeks,

103 were attacked, or 48·6 per cent.; whilst of 820, who took quinine irregularly and for periods from 1 to 10 weeks, 464 were attacked by malaria, or 56·5 per cent.

It should be here noted that in the surrounding villages, as well as in the whole of Attica, the malaria was very severe during the period under review.

The average amount of quinine consumed by each inhabitant, undergoing the preventive and curative treatment, was 15·6 grammes.

With regard to fatal cases of malaria, in the village of Marathon there died during the summer of 1907 one child of one year old, of pernicious spasmodic fever, and one girl of seventeen years of age, of blackwater fever. In the village of Bey, a child, aged 4 years, died of the latter disease. None of these children had undergone our preventive treatment. We have no information as to the number of deaths from malaria in the summer of the year 1906, but in 1905, seven died of that disease in the village of Marathon.

We hope that we shall be able to continue our work at Marathon under better auspices in the coming summer, when we shall have the advantage of the experience hitherto acquired.

The whole expenditure incurred in the six months' work at Marathon, amounted to Drs. 5.715.65 (£210), of which salary and travelling expense of the doctors, rent and sundry expenses connected with the staff, accounted for Drs. 3.548.70. The purchase of quinine, drugs, &c., Drs. 1.897.25, Petroleum and sundries Drs. 239.20.

The amount expended averages Drs. 3.70 per head for the six months, or less than that expended by the Italian Red Cross Society (which in 1901 amounted to fr. 11.25 per head) and the Austrian Government (Kr. 9.50 in 1903 and 1904).

Besides the sum expended at Marathon and the expenses on the two medical Congresses and the lecturing tour, a further amount of Drs. 6.820 (£250) was accounted for by the printing of the above-mentioned Statistics of the League, the detailed instructions regarding the prevention of malaria, and of the under-mentioned appeal on the part of the League.

The funds, which rendered possible the work of the League, were supplied by philanthropy.

The Committee formed in England, on the initiative of Professor Dr. Ross, under the presidency of Sir Alfred Jones, and which H.R.H.

Princess Christian graciously condescended to favour with her patronage, contributed a sum of £740, collected in Egypt and England.

Notwithstanding the fact that the thanks of the League were conveyed at the time by the President to the English Committee, we take this opportunity of again expressing our deep gratitude to all those friends, who, through their subscriptions so largely contributed to the success of the campaign, the chief object of which is to free our country from this age-long scourge, which has during so many centuries been the source of incalculable harm to the Greek nation.

Before concluding this Report, we must add a few words in connection with the Bill concerning quinine, which was drawn up and submitted to the Government two years ago through the instrumentality of the League.

In view of the fact that the quinine offered for sale, in the remoter parts of the Kingdom, is not only often of bad quality, but is also sold at a high price, and is further taken without any method by the peasants (who, owing to the frequency of the attacks of the disease, do not always consult a doctor, but treat themselves), the League drew up and submitted to the Chamber a bill providing for the undertaking of the sale of quinine by the Government.

In order that the Government, the Press, and Public opinion in general should be fully informed on the subject, the League issued and distributed 6,000 copies of an appeal (in March, 1907) in which it exposed the harm done by malaria, and, at the same time pointed out the remedies against the evil, chief amongst which were the draining of the pools and small marshes in the neighbourhood of dwellings and the introduction of a monopoly of quinine.

In this appeal there were published at the same time: (1) a report on the combating of malaria in Greece, sent us at our request by the Liverpool School of Tropical Medecine; (2) the Bill concerning quinine, with the report relating thereto, and, (3) a translation of the Italian laws concerning the combating of malaria.

The Committee of the Chamber, to which the Bill was forwarded, effected certain alterations therein, in conformity with the Italian system of selling quinine, and the amended Bill was passed in its last reading on December 15th of last year, becoming a law of the Kingdom.

According to this law, the Government has the right to procure and to sell any salt of quinine, which may be designated by the Board of Health. The quinine will be ordered through the Ministry of Finance on the basis of tenders (except if it is purchased from a foreign Government) on terms to be arranged on each occasion by the Board of Health. The limit for each order is fixed at two years.

The sale of quinine will be effected through the Chemical Laboratory of the Ministry of Finance, the Public Treasury, the Post and Telegraph Office, the Public School Teachers and by other public Offices, in virtue of a Royal decree.

The State will sell quinine at cost price, and a reasonable profit will be allowed to retailers. In the case of the sulphate and bisulphate of quinine, the law fixes the price per gramme for retail sale at a maximum of 10 lepta. The prices of other salts will be fixed by Royal decree as occasion arises.

This law does not prohibit the free import and sale of quinine, but quinine so imported will be chemically analysed before its entry into the country.

Penalties are appointed for the sale of State or other quinine at a higher price than that fixed, for adulteration of or the sale of adulterated quinine, or for smuggling quinine into the country, as well as for selling the article under weight.

The law further obliges those demes which suffer severely from malaria to enter in their budget an amount sufficient for the purchase of State quinine for the free supply to the indigent.

The above is a succinct account of the action of the League during the past year. As regards the future, the first item in the programme is the resumption of the campaign at Marathon for a series of years, and, subsequently, by the convocation of more doctors at the capital, and by means of lectures in other towns of the Kingdom, to contribute to the dissemination of the latest ideas regarding the prevention of malaria, and, to sum up, by means of suitable action with the Government and the municipalities together with the large landed proprietors and the public in general, to aim at the application of measures for the drainage of the large number of large and small marshes which cover our country.

This difficult work, however, imperatively calls for the co-operation of philanthropy.

TABLE A.—ANNUAL NUMBER OF DEATHS FROM MALARIA IN THE TWELVE CHIEF TOWNS OF GREECE

Towns	1899	1900	1901	1902	1903	1904	1905	1906	1907	Total number of deaths from Malaria	Average per year of Malaria deaths	Population	Rate of deaths from Malaria per 10,000 inhabitants	Yearly average of deaths from all causes	Rate of death from all causes per 1,000 inhabitants	Rate of deaths from Malaria per 100 deaths of all causes
Athens	40	59	78	45	56	46	57	51	71	503	56	174,430	3·59	3,582	22·99	1·56
Piraeus	30	27	24	22	22	14	24	18	13	194	22	74,583	3·33	1,397	21·25	1·57
Patras	40	39	39	37	31	19	40	17	31	293	33	37,724	8·72	965	25·52	3·41
Syra	0	3	3	0	1	2	1	0	1	11	1	17,809	0·51	600	30·77	0·16
Triccala	19	20	28	29	25	21	38	24	42	246	27	18,132	14·72	402	21·14	6·71
Corfu	2	26	17	8	2	7	4	5	2	73	8	29,032	2·84	665	23·57	1·20
Volo	23	19	10	10	43	47	115	47	96	410	46	23,563	21·89	490	23·27	9·36
Larissa	27	22	23	12	32	35	41	29	41	262	28	18,014	16·99	372	21·79	7·52
Zante	11	26	34	15	9	10	7	4	3	119	13	13,580	9·20	326	23·08	3·98
Calamata.....	15	16	14	14	16	34	51	12	21	193	21	15,397	13·38	336	21·40	6·25
Pyrgos	19	36	28	29	33	24	22	19	21	231	26	13,690	19·48	353	27·19	7·36
Tripolis	6	10	2	5	10	3	2	2	8	48	5	10,789	4·67	238	21·38	2·10
Total	232	303	300	226	280	262	402	228	350	2,583	286	446,743	9·80	9,726	23·61	4·33

TABLE B.—Number of Deaths in each Month during the Year 1907

Towns	Jan.	Feb.	Mar.	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Total
Athens	2	3	2	2	4	5	13	12	8	8	9	3	71
Piraeus	0	1	0	0	0	2	2	4	0	1	2	1	13
Patras	3	1	1	0	0	2	8	4	2	5	4	1	31
Syra	0	0	0	0	0	0	0	0	0	1	0	0	1
Triccala	0	0	1	0	0	2	5	8	6	8	10	2	42
Corfu	0	0	0	0	0	0	0	1	0	1	0	0	2
Volo.....	1	1	2	3	2	14	23	14	9	8	11	8	96
Larissa	1	1	1	0	0	6	3	6	7	11	3	2	41
Zante	1	0	0	0	0	0	2	0	0	0	0	0	3
Calamata.....	0	0	0	0	1	6	5	3	3	1	1	1	21
Pyrgos	0	2	1	1	1	3	3	2	2	2	3	1	21
Tripolis	0	0	0	0	0	0	0	1	2	2	0	3	8
Total	8	9	8	6	8	40	64	55	39	48	43	22	350

A PECULIAR INTRALOBULAR CIRRHOSIS OF THE LIVER PRODUCED BY THE PROTOZOAL PARASITE OF KALA-AZAR

BY

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In my report on Kala-azar, published in 1897, I described a dropsical form of the disease, which appeared, in part at least, to be produced by cirrhosis of the liver, which was found in one case post mortem. More recent experience has shown this complication to be commoner in the more chronic sporadic form of kala-azar, which is so frequent in Calcutta, than it was in the Assam epidemic disease. These cases, however, were very difficult to verify by finding the parasite by spleen puncture on account of the organ receding into the abdominal fluid before the point of the needle, although the characteristic history, greatly enlarged spleen and leucopenia, left no doubt in the mind of an experienced physician regarding the true nature of the cases. Some degree of fibrosis of the liver was described in my first 1897 report, while about a year ago a post mortem on a chronic case of kala-azar, which died with all the classical symptoms of cirrhosis of the liver, has enabled me to study the advanced stages of the affection. I, therefore, propose in the present paper to give a somewhat fuller account of the affection than the note in my work on Fevers in the Tropics, in which the photograph of a kala-azar patient suffering from this type of cirrhosis of the liver is given (opposite page 67). The following are the notes of the case referred to above.

CHRONIC SPORADIC KALA-AZAR TERMINATING WITH CIRRHOSIS OF THE LIVER

The patient was a Hindu male, aged about 30. He had suffered from enlarged spleen with frequent attacks of fever on and off for five or six years. During the last six months he had taken country liquor, but not in excess. He was in hospital for 35 days before his death, during which time he suffered from ascites with enlargement of the liver and spleen, and persistent diarrhoea, and was greatly emaciated and anaemic, and had a troublesome cough. No malarial parasites could be found in his blood, and he only occasionally had a slight rise of temperature. The disease was diagnosed as kala-azar.

A post mortem was performed and the following conditions noted. The body was extremely emaciated, and the subcutaneous fat was very scanty. The peritoneal cavity contained 30 ozs. of clear fluid, but there was no oedema of the legs, the dropsy having decreased with the diarrhoea while in hospital. The pleural cavities showed some fibrous adhesions, but the lungs were crepitant throughout, although slightly congested at the bases. Both the parietal and visceral pericardium showed extensive haemorrhages (these being common in kala-azar in various positions). The heart muscle was somewhat pale, but firm. The stomach showed petechial haemorrhages in the mucous membrane. The small intestines showed a few points of haemorrhage due to the bites of a small number of anchylostomata (such as are found in 75 per cent. of post mortems in natives in Calcutta), but were quite free from ulceration. The large intestine showed some pigmented scars, while the mucous membrane of the lower part was congested and oedematous. The liver weighed 29 ozs. (the body weight was only 60 lbs.). The surface was perfectly smooth, and of a greenish brown colour. It cut very firmly, and its substance could not be broken down by very firm digital pressure. The gall bladder was healthy. The spleen weighed $12\frac{1}{2}$ ozs., having become much diminished in size during the continuance of the diarrhoea. The capsule was wrinkled, and on section it was a brownish-red colour and very firm, owing to excess of fibrous tissue. The kidneys were healthy except that the capsule stripped with some difficulty, but left a smooth surface. The brain was healthy.

MICROSCOPICAL EXAMINATION

The protozoal parasites of kala-azar were found in large numbers in the bone marrow, spleen and liver, being mostly of the small size seen in chronic cases of the disease. In the liver they were found with an oil immersion lens in the endothelial cells of the capillaries between the columns of hepatic cells in specially prepared specimens. With haematoxylin and eosin only very fine dots could be detected in this position, being the nuclei of the parasites, closely resembling pigmentation, for which I think I must have sometimes mistaken them in my original Assam investigation in 1896. The persistence of the parasites in the advanced cirrhotic stage of the organ is remarkable, and leaves little doubt that they are the cause of this peculiar intralobular cirrhosis.

The general appearance of the liver under a low magnification (Zeiss A. Oc. 2) is shown in fig. 1 of Plate I. To the left is seen a portion of the capsule at the site of an extensive fibrous band, but it will be observed that there is no marked depression of the surface such as produces the hobnail appearance of the common atrophic cirrhosis of the liver. The capsule itself also shows but very slight thickening. The most striking feature is the universal distribution of the cirrhotic process throughout the liver lobules, so that the hyperplastic connective tissue widely separates each column of epithelial liver cells, and, indeed, makes up the greater bulk of the lobules from the portal to the hepatic venules. A careful study of a number of sections showed that there is extremely little alteration in the general arrangement of the liver lobules, which retain to a great extent their shape and size, although the intralobular veins are somewhat less prominent than usual. There is distinct cellular and fibrous increase around the portal interlobular veins, but not extending far round the circumference of the liver lobules as a rule, so that the organ is not cut up into small areas of hepatic substance by complete circles of fibrous tissue, as in atrophic cirrhosis. This explains the absence of the typical yellow lobulated appearance to the naked eye on section of the organ, which is so characteristic of hobnail liver, and has probably led to the new form having been frequently overlooked, especially in its less marked degrees. The perilobular portal tissue shows a few well-marked bile-duct-like double columns of some-

what cubical epithelial cells, but these are nothing like so numerous as in hypertrophic cirrhosis of the liver, being, indeed, scarcely more evident than in the atrophic form.

Fig. 2 of Plate I shows a portion of a liver lobule under a higher magnification (Zeiss A, Oc. 2), and includes some of a periportal fibrous band. The very great increase of the intercellular connective tissue of the lobule will be at once apparent, the epithelial cells forming barely half of the area of the lobule, each column of liver cells being separated from the next by an extensive layer of fibro-cellular connective tissue. In places the liver cells contained much yellow pigment derived from broken down red cells—anaemia being a marked symptom of the later stages of kala-azar, although frequently only slight in degree in the first few months of the disease, much less so than in true malarial fever of any duration. With this exception the surviving liver cells have a fairly healthy appearance, and stain well. The connective tissue between the liver cells is partly fibrous, but chiefly consists of small round cells together with a considerable number of larger epitheloid-like ones, some of considerable size. It is the latter which were found to still contain the parasites of kala-azar in specially stained sections, and they are doubtless the enlarged endothelial cells of the capillary vessels, which S. R. Christophers first described as containing the human stage of the kala-azar parasite.

This distribution of the organism at once furnishes the key to the peculiar position of the connective tissue proliferation. These minute protozoa multiply in the endothelial cells, and on reaching their full size some of the cells rupture, scattering the parasites into the blood stream, where they are found in comparatively small numbers, in the polynuclear leucocytes more particularly. These bring them back again to the liver, spleen and bone marrow, where the cycle is repeated. When this process continues for a number of years, as in the chronic form in which alone I have seen cirrhosis of the liver supervene, it is not surprising that eventually the constant irritation of the parasite causes proliferation of the connective tissue around the capillary vessels throughout the liver lobules, and to a less extent in Glisson's capsule, around the portal radicles, and so produces the condition above described. This universal thickening round the capillary vessels of the liver causes both an extensive

atrophy of the hepatic cells and also must considerably retard the circulation of the portal blood. It thus produces the marked ascites, which, although the fluid accumulates much less rapidly than in atrophic cirrhosis, yet is a formidable and not infrequently fatal complication in these unfortunate people, worn out as they are by years of fever, and reduced to an extremely debilitated and emaciated state.

FREQUENCY OF INTRALOBULAR CIRRHOSIS OF THE LIVER IN KALA-AZAR

Among 48 post mortems I have performed on sporadic kala-azar in Calcutta in the last few years, marked cirrhotic changes were present in the liver in four; while in seven more, slighter degrees of fibrosis were met with. The latter number is certainly too low, for in half the cases the exact consistence of the organ was not recorded in the post mortem notes, and this degree is very easily overlooked. On the other hand, the liver was noted to be softer than normal in nine cases, so even slight fibrosis is very far from being constantly met with. This is due to the extreme variation in the duration of this fever, namely, from a few months to five to ten years. In my work on Fevers in the Tropics a table of the degree of enlargement of the liver in different stages of kala-azar is given, from which it appears that marked enlargement of the liver is rarely seen before the end of six months fever, while the cirrhotic condition usually only appears after several years illness.

MALARIAL CIRRHOSIS OF THE LIVER

In view of the fact that until the last few years kala-azar has always been classed as 'malarial cachexia,' the discovery of the above-described form of cirrhosis due to kala-azar raises the question as to how far descriptions of malarial cirrhosis of the liver may have been based on cases of kala-azar erroneously diagnosed as malarial. In this connection it is worth recording that in five years pathological experience at the Medical College, Calcutta, I have only once met with a case of undoubted malarial cirrhosis of the liver, in which the microscopical picture of uniform extensive thickening of the perilobular connective tissue with much black pigment in its lymph spaces, and to a less extent in the intracellular tissue throughout the

liver lobules, was so characteristic that it could not possibly be overlooked. My impression, therefore, is that a true malarial cirrhosis of the liver does occur, but that it is certainly decidedly rare even in highly malarious Lower Bengal, for a large proportion of our cases come from the unhealthy districts surrounding Calcutta.

I may also mention that typical atrophic cirrhosis is extremely common in Bengal, more so even than in Europe, although it is certainly not as a rule due to alcohol. Major O. W. Sutherland has also recorded a similar experience in the Punjab. Thus in five per cent. of over 4,000 post mortems in Calcutta cirrhosis of the liver was found, although 40 per cent. of the deaths were from typically tropical diseases, such as cholera, &c. If these are excluded the percentage rises to between 8 and 9 per cent. of the deaths. On the other hand, in Berlin Forster found cirrhosis of the liver in but 1 per cent. of 3,200 post mortems. This very important subject, however, is beyond the scope of the present paper.

SUMMARY

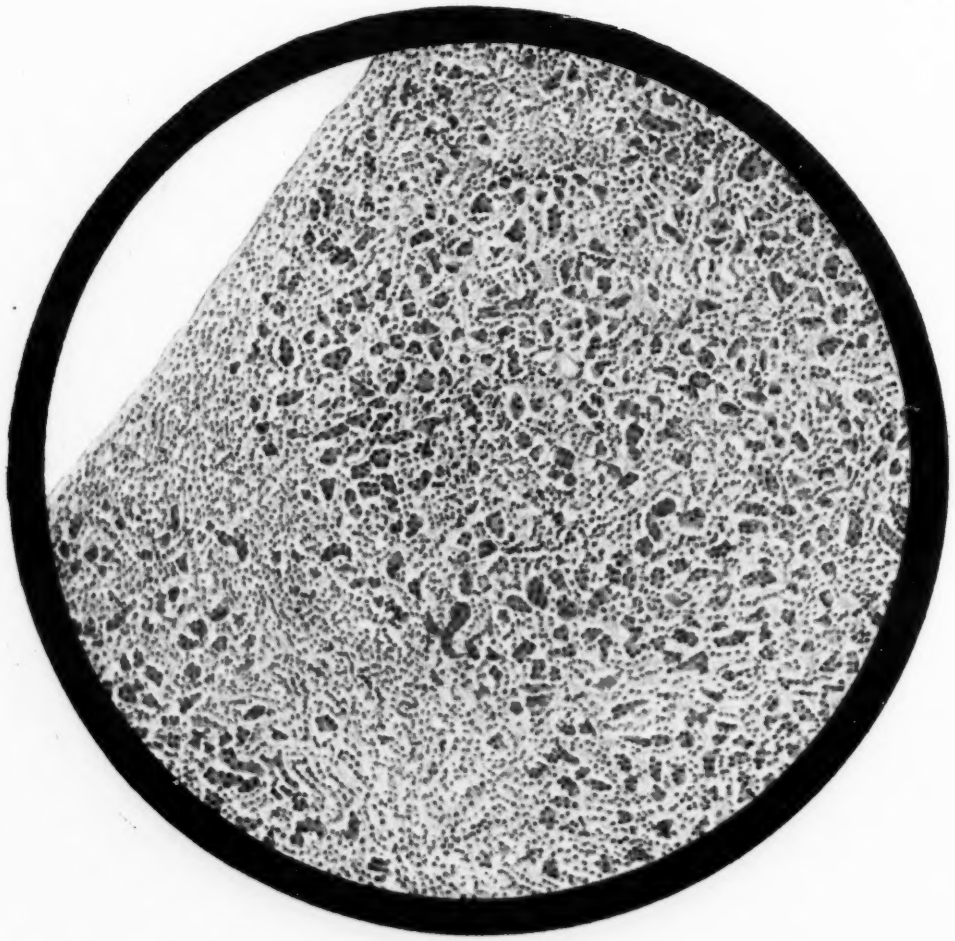
1. The most chronic cases of kala-azar not infrequently terminate their course with ascites due to cirrhosis of the liver.

2. The cirrhosis is of a peculiar intralobular type of uniform distribution and with a smooth surface to the organ.

3. It is due to the protozoal parasite of kala-azar, which may be found in the liver and other organs after death.

4. This form of cirrhosis of the liver is much commoner in Lower Bengal than a true malarial cirrhosis, with which it has probably hitherto been confused. It is, however, much less common than atrophic cirrhosis due to unknown causes.

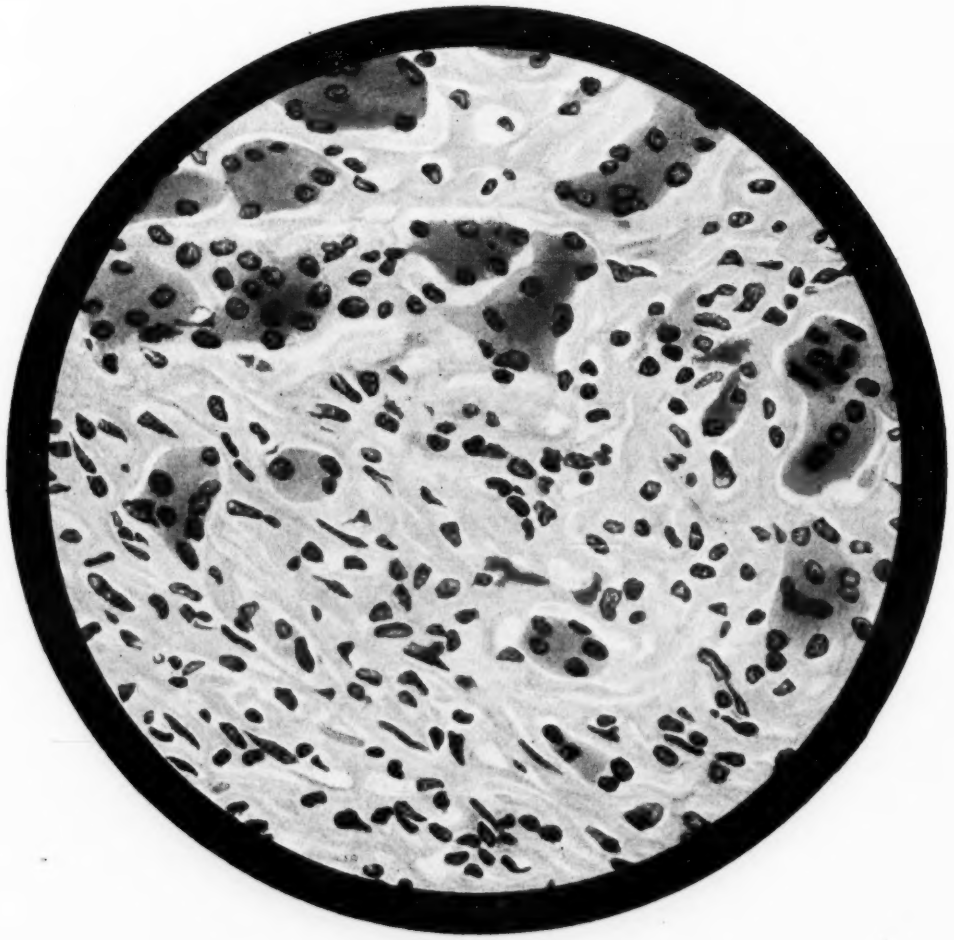
1. Zeiss A Oc. 2.



INTRALOBULAR CIRRHOSIS
OF KALA-AZAR.

Fig. 1

2. Zeiss D Oc. 2.



INTRALOBULAR CIRRHOSIS
OF KALA-AZAR.

Fig. 2

PLATE I

WHAT IS '*SCHISTOSOMUM MANSONI*' SAMBON 1907?

BY

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(*Received for publication* 21 *March*, 1908)

About a year ago Dr. SAMBON, of the London School of Tropical Medicine, startled the scientific world interested in human parasitology by the creation of a new species of blood fluke, *Schistosomum mansoni*, which he stated had hitherto been confounded with *Sch. hæmatobium* (1907a, p. 117). A suggestion to this effect had been made as far back as 1903 by Sir PATRICK MANSON. SAMBON's new species was thus readily adopted by MANSON in the new edition of his 'Tropical Diseases' (1907, p. 660). From the West Indies there had come information which seemed to corroborate Dr. SAMBON's views (HOLCOMB, 1907). Later, the author gave a somewhat fuller account of the new species (1907b, p. 303), and quite recently, he mentioned its existence as a fact in a paper 'On the Part played by Metazoan Parasites in Tropical Pathology,' read before the Society of Tropical Medicine and Hygiene, London (1908, p. 29f). In this paper and in the ensuing discussion repeated allusion was made to the views hitherto held with regard to Bilharzia and Bilharziosis by the workers in Egypt in general, and particularly by myself. It was hinted that we had not recognised the differences between two easily distinguishable species. If Dr. SAMBON's view were correct, all of us who have devoted attention to the subject, would have indeed been wandering in the dark since the time of BILHARZ himself, fifty-seven years ago. Since such a charge has practically been made I feel it necessary to take up the defence. I may as well at once say that when in London two years ago I dropped some well-meant hints of warning to be cautious, whether to Sir PATRICK MANSON or to Dr. SAMBON I do not remember. I am sorry that these hints have not been heeded, for if so the present disagreeable discussion might not have become necessary.

Being at present fully occupied with some other work I must limit myself to the discussion of some points of primary importance; but it is possible that I may take an early opportunity of returning to the subject in more detail. Speaking quite generally, I may say even now that those of us who have seen anything of Bilharziosis in Egypt are convinced that the scientific problem it offers is more complicated than Dr. SAMBON seems to imagine; it is a problem which will require a long and close collaboration of the Anatomist, the Pathologist, and the Helminthologist before it may be considered as solved in every detail. There is, further, one thing which can not be too strongly emphasised at the very outset, and this is that any theory, be it ever so cleverly based on the biology of the parasite, must be wrong if it contradicts the facts supplied by the Anatomist and the Pathologist; and also that any other theory, however plausible its explanation of the anatomo-pathological observations, can not represent the truth so long as it is irreconcilable in any detail with the biology of the parasite. I may mention in passing that several theories recently brought forward as explanations of the aetiology of certain human diseases caused by worms are open to the latter objection; the theory of SAMBON is the latest of these, and a very interesting one it is, not perhaps so much for the arguments by which, but on account of the manner in which it is supported.

In order to make the purport of what I have to say hereafter quite clear, I will state that I do not consider it my task to prove whether or no there exists a *Schistosomum mansoni.* Among scientific workers it is a good custom that anyone who believes he has made a new discovery also takes the trouble to prove it; it is not customary among scientists to assert something and call for the help of others to establish it. In the case which I am about to discuss, it is Dr. SAMBON who, acting upon a suggestion of Sir PATRICK MANSON, formally published *Schistosomum mansoni* as a new species. After the usage generally adopted in science, the merit of the discovery is his when the discovery is right. But with him also must rest the responsibility of bringing forward all the evidence which may be reasonably demanded in support of it. Dr. SAMBON, indeed, supports his action by a certain amount of evidence; but he is obviously aware himself that, especially, its zoological part (i.e. the possibility of distinguishing the adult forms) is

practically nil. He therefore concludes his answer to the various objections made in the discussion by expressing the hope (in a form, by the way, which I fail to appreciate) that I would soon be able to provide that description of the adult forms which he himself was unable to produce (p. 46). I am sorry that I cannot accept the part thus assigned to me. If Dr. SAMBON had not sufficient material to demonstrate beyond doubt the specific independence of *Sch. mansoni*, he might with advantage have postponed the publication of the name until such necessary material was available. Since he has gone so far as to publish the name, and thereby implicitly claims to have made an important discovery, I think that it is incumbent upon him, and not upon me (or any other), to do the work of supplying such proof as the rest of the scientific world may ask. As I have already said, I cannot consider it as my legitimate task to prove or disprove the existence of a '*Sch. mansoni*.' What I propose to do is to point out the inadequacies of Dr. SAMBON's theory. Doing this, i.e., giving the reasons against SAMBON's theory, amounts to practically the same thing as giving the reasons for the views held by me with regard to some fundamental items in the biology of *Sch. hæmatobium*. I am not displeased to have this opportunity: for the rest, every reader is free to form his own judgment.

The reasons which lead Dr. SAMBON to assert the existence of a separate species, '*Sch. mansoni*,' are three: a zoological, a pathological, and a geographical one. The first is afforded 'chiefly' by the ova. 'In *Sch. hæmatobium* the eggs are more or less lanceolate, and provided with a short, straight, terminal spine; in *Sch. bovis* they are spindle-shaped, and provided with a short, terminal, heart-shaped spine; in *Sch. japonicum* they are ovoid, and have no spine; and in *Sch. mansoni* they are oval and provided with a stout, lateral spine' (1908, p. 31). The adults producing the two varieties of eggs are as yet indistinguishable. Dr. SAMBON 'had the opportunity of examining several specimens collected at post mortems in Egypt and Uganda.' He 'noticed that whilst the majority of female worms contained within their uterine tubes the characteristic ova of *Sch. hæmatobium*, with a short terminal spine at the posterior extremity, two presented lateral-spined ova. These had been removed from the gynaecophoric canal of males differing in no appreciable way from those clasping the more common kind.

Unfortunately, the material at hand was so badly preserved that it precluded any study of comparative anatomy' (1907b, p. 303).

The second specific character of *Sch. mansoni* is, according to Dr. SAMBON, given in its 'different anatomical habitat' and its 'specific pathogenic action' (1908, p. 32). '*Sch. mansoni* does not affect the genito-urinary organs, its ova are eliminated solely by way of the intestine; they are never found in the urine. The patients harbouring this parasite suffer from a haemorrhagic enteritis, but they never present haematuria' (1907a, p. 117). The third reason for assuming the existence of a *Schistosomum* different from *Sch. hæmatobium*, is found by Dr. SAMBON in the peculiar geographical distribution of *Sch. mansoni*. According to the data published in literature, the new species is 'probably' alone present in the West Indies, for endemic haematuria is unknown there. The same may be said with regard to the Congo Free State, where careful recent investigations have shown the absence of haematuric bilharziosis and the frequency of a rectal infection, in which the ova of the parasite bear invariably a lateral spine. In the Cape Colony, on the other hand, haematuria is very common; HARLEY, BROCK, and others working in those districts state in their articles on the subject, that they never encountered the egg with the lateral spine. In Egypt, both *Sch. hæmatobium* and *Sch. mansoni* are found side by side, but the former appears to be far more prevalent, and is certainly more in evidence, owing to the haematuria to which it gives rise. That is probably the reason why the two forms have been confounded, and the scarce, laterally-spined ova looked upon as abnormal and distorted (p. 32). SIR PATRICK MANSON, on page 660 of his text-book, shortly states that BILHARZ in 1851 noted the presence of a *Schistosomum* producing lateral-spined eggs, but confounded it with *Sch. hæmatobium*.

We will now analyse these reasons given by Dr. SAMBON somewhat in detail. I begin with the zoological. It is the most important; for the foundation of a new species is, to put it briefly, in the first place a 'zoological act.' In order to establish a new species safely it is necessary to point out constantly present and, if possible, easily recognisable zoological characters by which it may be distinguished from related forms. The less constant and the less definite the characters of a

presumed new species are, the more it is contestable from the zoological standpoint. The characters themselves must, first and foremost, be derived from the adult stages. With regard to this point, Dr. SAMBON's evidence is nil. He has examined some badly preserved specimens, but the males showed no difference at all from those of *Sch. hæmatobium*, and the females differed in the shape of the egg only. To this latter we shall return later; speaking of the adults, I may state, from a general point of view, that I would not, *a priori*, consider it as a serious objection to Dr. SAMBON's views if there really were no marked anatomical differences between the adults of the supposed two species. There are some few cases known in which certain forms resemble each other to such an extent that they might well be representatives of the same species, did not other factors—such as they are known to us at present—seemingly exclude the possibility of the forms being the same thing. Dr. SAMBON, in order to make the absence of all distinctive characters of his new species appear less weighty, dwells at some length on two cases in which, after a long and tedious comparison of many adult specimens, I have myself come to similar conclusions. It would lead me too far to discuss these cases in detail here. I will only remark that one of them has no bearing on the case at present under discussion, inasmuch as the forms in question show differences which, though slight, are yet sufficiently pronounced to enable any expert to distinguish the respective forms as easily as he may distinguish *Sch. hæmatobium* and *Sch. bovis*. In the second case, a parasite was found to inhabit several mammals, but to be entirely absent from birds, in Europe; whereas a similar form, in North Africa, could never be found in the same mammals, but was present in birds which never visit Europe. In this case, I of course depend upon the facts available at present, and it is very probable that a comparison of a larger supply of new material (my own investigations were made 15 years ago) will reveal structural differences here also. But unless the difference be cleared up by new observations I feel compelled to consider the respective forms as different, in spite of their apparent structural identity. However, Dr. SAMBON, or anybody else, is fully at liberty to show that the premises on which my opinion is based are erroneous. If he succeeds in showing this by irrefutable facts I shall certainly be the first to change my opinion.

But, before doing so, I want to hear facts, just as in the present article I am about to point out a number of facts which are irreconcilable with Dr. SAMBON'S theory. He assures his audience that 'certainly there were more and better reasons to separate *Sch. mansoni* from *Sch. hæmatobium*' (1908b, p. 46) than there were in my two cases referred to by him. We shall see how far this is true.

Dr. SAMBON first pretends that the other known *Schistosoma* species do not show any marked differences in their adult stages. 'The *Sch. bovis*, for instance, resembles the *Sch. hæmatobium* so closely that, indeed, it would be very difficult for anyone to point out any marked difference between the adult forms of the cattle parasite and those of *Sch. hæmatobium*' (1908b, p. 46). To this I have to reply that Dr. SAMBON is mistaken. To 'anyone' who actually has some helminthological knowledge a single glance with the naked eye will suffice to tell *Sch. hæmatobium* from *Sch. bovis*, and a good pocket lens will suffice to differentiate *Sch. japonicum* from *Sch. hæmatobium*. There are, in addition, quite well-marked internal differences which Dr. SAMBON might have known had he consulted the latest description given of *Sch. bovis* by Leuckart (1894, p. 470f), or the short review I gave of KATSURADA's paper on *Sch. japonicum* (1905a). The fact that Dr. SAMBON is not apparently aware of the existence of these differences is in itself a poor reason for the statement to his audience that they are really absent. As a matter of fact, in no case does the differentiation of any of the species of *Schistosomum* hitherto described, whether affecting man or animals, depend on the form of the egg alone. Since the various Schistosomes affecting animals are not mentioned by Dr. SAMBON, I will not refer to them any further in this discussion.

Thus, Dr. SAMBON is not able to produce any distinctive anatomical character of the adult *Sch. mansoni*. There remains only the egg. It is a well-known fact that many, but by no means all, species of parasites may be recognised from their eggs. If this is the case, the aspect of the egg is one of the distinctive characters of certain species. I do not, however, at present remember one single case in which two species of parasitic worms acknowledged as independent differed solely by their eggs. The fact is easily comprehensible. If I cannot tell whether two specimens I have before me are individuals of one species or

individuals of two species, I cannot tell either whether slight differences I observe in their eggs are specific characters or not. If I so desire, I may assert that there are two species; but, in that case, others will certainly demand proofs of such a statement. Dr. SAMBON pretends that the two shapes of the egg found in association with *Sch. hæmatobium* belong to two different species, but I cannot see that he can possibly prove this zoologically without finding distinctive differences between the adults. For the proof must consist in showing that one form of egg is constantly connected with a certain anatomical structure, and the other form as constantly connected with another anatomical structure of the adults. Until this is done I am afraid that *Sch. mansoni* will find little approval with zoologists, in spite of Dr. SAMBON's contention that 'to zoologists the characters of the ovum should suffice for the determination of a new species' (1908a, p. 31).

The remarkable difference in the position of the spine of the egg of *Sch. hæmatobium* has long attracted the attention of observers, the majority of whom considered the egg with the end spine as the normal, and that with the side spine as abnormal. Various attempts have been made to explain the formation of the latter. Dr. SAMBON refers to these theories, but in a rather peculiar manner. He particularly mentions FRITSCH, 'who had described certain differences in the genital tract of the female, but was under the impression that the females containing the lateral-spined ova belonged to the same species as those containing terminal-spined ova. He therefore explained the difference by abnormality. FRITSCH's explanation was obviously wrong, but his description was perfectly correct' (1908b, p. 46). I should like to know in what way Dr. SAMBON has obtained the evidence for the concluding part of this statement. He has said that the two females he had an opportunity of examining were so badly preserved that any study of their anatomy was precluded. How, then, does Dr. SAMBON know that FRITSCH's description was 'perfectly correct'? I doubt whether he has at all read that author's original article (it is, unfortunately, not accessible to me at present); he has certainly not read the later descriptions of LORTET and VIALLETON, LEUCKART and myself, in the latter two of which FRITSCH's statements with regard to the point under discussion are refuted as incorrect.

I will not tire the reader by a long anatomical description of the structures at issue; suffice it to state that up to this day I have personally found only one type in the structure of the internal genital organs of the female, although the uterus may contain in one specimen ova with a terminal, and in another specimen ova with a lateral spine. The position of the spine does not depend upon a preformed difference in the internal structure (which, of course, changes its shape somewhat with the contractions of the body), but on the relative position of the egg during the process of its formation in the ootype. I have tried to show this in a diagrammatic drawing which has recently been copied in various books on Bilharziosis; I may mention in passing that in this figure the lateral-spined egg is placed unusually steep; I have in the meantime come across worms in which the axis of the egg lay almost at right angles to the axis of the ootype. Dr. SAMBON ignores the existence of this drawing as well as the descriptions of LEUCKART and myself; I should like to submit that he will have to account for them if he wants to maintain *Sch. mansoni* as an independent species.

On the whole, the zoological characters of the new species are as vague as they can possibly be. Dr. SAMBON is himself aware of that and refers to a case where, in one instance, ornithologists have based a new species solely upon the character of the egg. I am not in a position to criticise the actions of ornithologists; but the fact that they find something justified is for me not in itself a reason to consider the same thing as justifiable also in helminthology. I would mention, by the way, that the new species of bird will certainly not be generally accepted unless it can be shown that the aberrant shape of the egg is reasonably constant.

The details thus far mentioned are in the main of a technical zoological nature. I should not have been compelled to enter upon them had not Dr. SAMBON tried to show that the foundation of *Sch. mansoni* was justifiable from the zoological standpoint. That it cannot be, will become obvious even to the non-specialist by another fact not mentioned by Dr. SAMBON. The fact is that in Egypt, the eggs of *Sch. hæmatobium* and '*Sch. mansoni*' may occur in one and the same individual female. This observation is now 57 years old and might have been known to Dr. SAMBON, had he studied the papers of those

authors whom he accuses of having failed to recognise an obvious fact. The observation is due to BILHARZ. It is true that BILHARZ did not yet know how to interpret those bodies which we now describe as lateral-spined eggs; but this is of no importance as compared with the fact, that once he found one of these enigmatical bodies in the anterior part of the uterus of a female, the posterior part of which was filled with the ordinary ova. That there was no mistake possible may be gathered from the circumstance that BILHARZ, on a later occasion, and after having discovered the same bodies in the tissues of the liver and the rectum, emphatically repeats that 'such a body was, though once only, but quite undoubtedly, found in the uterus of a female worm, the posterior part of which contained the normal ova' (BILHARZ, 1852, pp. 74 and 75). Besides, BILHARZ has proved too careful an observer to admit of any mistake on his part; as a matter of fact, many a recent 'discovery' with regard to Bilharzia and Bilharziosis may be found described in his paper when one takes the trouble to read it.

If my memory does not quite fail me, I have in the course of years, myself seen several similar females; but considering the occurrence of both shapes of eggs in the same individual as anything but new, and not foreseeing either the importance the specimens would one day gain, I have not separated them from the rest, and it is quite possible that one or the other may be found in the material which I have sent away from here to various places. I very much regret that at the present moment I cannot produce a specimen. It is a curious fact, of which we shall have to speak again later, that the portal veins very often contain only males; the worms within recent years found at the post mortems in the Kasr el Aini Hospital, and kindly left to me by Dr. FERGUSON, were almost exclusively males; in one of the last cases, e.g., there were 64 males but not a single female. I have, however, no doubt that sooner or later I shall be able to establish the accuracy of BILHARZ's observation by the production of an actual specimen.

The occurrence of terminal-spined and lateral-spined eggs in one and the same individual worm is one of the fundamental facts on which my views rest; I wonder how Dr. SAMBON will explain it by his theory.

I have said above that, a priori, a great structural similarity of the adult stages would not necessarily be a proof of there being only one species. If, on the other hand, I am asked to acknowledge a specific difference between *Sch. hæmatobium* and *Sch. mansoni*, in spite of their great internal resemblance, I certainly expect that the other proofs in favour of the existence of a separate species will be absolutely clear and stringent. We will now see how these parts of Dr. SAMBON's evidence stand an earnest scientific test.

There are numerous cases where closely allied parasites (of man or animals) show marked differences with regard to their special habitat in the body of their host; the lesions they produce will then show a peculiar localisation. Closely allied species may further differ in their geographical distribution which is indicated by the geographical occurrence of the respective lesions. It is, therefore, a priori, imaginable that the localisation and the geographical occurrence of certain symptoms may in certain cases be a valuable support for the distinction of the species which cause them. A different question is whether variations observed in the localisation and the geographical distribution of certain symptoms may be used as proof that they are due to different species of parasites. In this connection I must point out that, quite generally speaking, observations of the alleged sort are, in principle, statistical. I do not underestimate the value which statistical observations may have under various circumstances; but it is a fact also that from the same statistics more or less opposite conclusions may be drawn according to the point of view from which they are looked at. I may add that the statistics themselves are by no means all of them equally reliable. On the whole, therefore, I think that it will always be wise to test statistical observations very carefully before considering what they seem to show, as an objective proof of some theory. An excellent example of the truth of what has just been said will be discussed towards the end of this article.

In the case which at present occupies us Dr. SAMBON uses statistical observations referring to the localisation and geographical distribution of certain lesions as additional proofs of the existence of a species of parasite which is zoologically utterly doubtful. Judging a priori, I would consider the species as established, notwithstanding, when the additional proofs were binding and did not leave any

visible gap. I am sorry to say that, from my point of view, Dr. SAMBON's proofs do not answer this description.

The second difference between *Sch. hæmatobium* and *Sch. mansoni* is said to be given in the different anatomical habitat, and the specific pathogenic action of the latter form. We will start with the well-established clinical fact that terminal-spined ova alone are voided from the bladder, whereas, lateral-spined are met with in the faeces. The conclusion generally drawn from this observation, and also brought forward in the discussion above mentioned by Sir PATRICK MANSON, is that the lesions of the bladder are caused by worms producing terminal-spined ova, whilst the almost identical lesions of the rectum are caused by worms producing lateral-spined ova; in other words, that the last-named ova appearing in the faeces are derived from the rectal lesions. In connection with this point, Sir PATRICK MANSON asked whether anybody had ever seen a lateral-spined egg in the urine. Nobody could answer in the affirmative; indeed, I do not remember myself to have specially noticed a lateral-spined egg in the urine. Thus far, observations agree very well; however, what I should like to point out is that even if I, or anyone else, had seen a lateral-spined egg in the urine, I would be unable to consider this as a fact of fundamental importance. To me it would appear as an accidental exception, due to accidental reasons, to the rule that the urine contains terminal-spined eggs only. One may examine the faeces of a thousand people without finding an apple-maggot, and in the faeces of the thousand and first there is one; the former observations show that maggots are not a normal appearance in human faeces; the latter observation does not at once demonstrate the contrary, but only shows that it is an occasional exception, the chief interest of which would lie in the question as to the conditions under which the exception occurs. As I have said, I would look at it from this point of view, should a lateral-spined egg some time be found in the urine.

Passing to the supposed causal connection of the lateral-spined ova with the rectal lesions, I must confess that up to a few years past I shared the opinion that the former were derived from the latter. I must state to-day that this was a mistake; in many cases the lateral-spined eggs do not come from the

rectal lesions. Desiring, some years ago, to make a drawing of a lateral-spined ovum, and having no faecal material at my disposal I took a papilloma of a preserved rectum and examined for ova. There were plenty of terminal-spined, but not a single lateral-spined could be discovered. New preparations made from other papillomata of the same rectum, gave no better results. Serial sections made of other recta showed similar conditions, in so far as sometimes terminal-spined eggs alone were found, sometimes both forms mixed. In no case, however, have I so far found, in the rectal wall, the lateral-spined eggs quite alone. The point most important in the present connection is that these observations leave no doubt that the vesical and rectal lesions so similar to each other in external appearance also contain one form of egg only. After the theory of Dr. SAMBON, this form is distinctive of *Sch. hæmatobium*. *Sch. hæmatobium* is, therefore, capable of producing rectal as well as vesical lesions; I see no reason why, under peculiar circumstances, it should not be able to produce, in one case, vesical lesions alone, and, in another case, rectal lesions alone. The question as to what these peculiar circumstances may be is certainly of great interest, but it is secondary to the fact that both forms of lesions may be produced by *Sch. hæmatobium*. '*Sch. mansoni*' is said by Dr. SAMBON to have 'a specific pathogenic action'; since *Sch. hæmatobium* may produce identical lesions the alleged specificity does not exist, or shows, at least, a very remarkable flaw.

We now come to a more important point. If lateral-spined ova do not occur at all, or occur in insignificant numbers only, in the rectal lesions it is impossible that such ova when they appear in the faeces can be derived from the rectal lesions. I had, in a number of cases selected entirely at random, found no trace of lateral-spined ova in the rectal papillomata; where, then, could the lateral-spined ova come from? Before advancing further I must mention several facts which, in addition to the occurrence of both shapes of eggs in one and the same individual, figure as arguments in my theory as to the nature and the significance of the lateral-spined eggs.

The place where the Bilharzia worms are, in post mortems, usually looked for, and most easily found, is the portal system. I have, since

1893, seen a good many of them; a fact which struck me from the beginning was their very different size. In certain cases, they presented about their normal dimensions; in others, they were markedly smaller, and in some, they hardly reached a third of their normal length. Another fact which sometimes very forcibly obtruded itself to the eye was that the specimens present in an individual case were, among themselves, of very much the same size, i.e., of about the same age. I still possess in my collection the material from one case, which consists, after specimens have been given away, and others have been used for examination, of 62 males, all varying in length from 3 to 4 mm. according to their somewhat different state of contraction. There are, in addition, females (though in fragments only) which must have measured from 5 to 6 mm. so far as their length is still determinable. I also remember another case in which the worms—males alone—presented two different sizes so distinctly that it was not difficult to separate them into two lots, each, of specimens about equal in size. On microscopical examination, all specimens proved to be sexually immature, and the degree of sexual development coincided about with their size. In many cases males were present alone; where both males and females were found they were still isolated; only in some two or three cases could a coupled pair be detected in the portal veins. The more advanced females contained one or a few ova in their uteri, all of them of the lateral-spined type, some of quite unusual shape. These observations only confirmed what had been seen and described by some former writers.

As a helminthologist I have not limited my investigations to the parasites of man, but have carefully and through many years, studied—anatomically and biologically—the Trematodes parasitic in animals. One result of these studies was that, very generally, Trematodes at the approach of their sexual maturity were found to form abnormally shaped eggs. In some most interesting instances the female genital apparatus was, owing to some malformation, found completely shut off from the male apparatus; there was no possibility for the egg-cells to become fertilized, but, nevertheless, the uterus was filled (in one case packed) with ova, all misshapen. In younger but normal specimens of the same species, the uterus contained more or less numerous normal eggs, but in front, there were, sometimes a

few, in other cases more, and in others again, crowds of the same abnormal eggs as had been seen in the specimens with the internal defects. In order to fully understand these statements one must, of course, have some knowledge of the anatomy and biology of the parasitic worms in general. I do not expect the ordinary medical man to have them, nor does he want them; but I strongly recommend studies of the sort to all those who indulge in 'formulating ideas' with reference to helminthological questions. Anyone would be laughed at if he tried to write a tale in a language of which he did not know the alphabet; but I might quote dozens of passages from modern papers on helminthological subjects which leave no doubt that the author did not know the significance of the terms he used.

Putting the facts observed in various species of Trematodes together with what had been seen by some earlier observers and myself in the young Bilharzia worms, I came to the conclusion that the lateral-spined must be abnormal eggs. I added that unimpregnated or isolated females would, perhaps, be 'unable to produce other than such abnormal eggs.' I do not claim that this interpretation is the correct one; but I daresay that it is based on a series of actual facts observed in the nearest natural relatives of the Bilharzia worms; in other words, that it is a quite well-founded 'conclusion from analogy.'

Dr. SAMBON, speaking of this theory of mine, refers to an observation of Dr. LEIPER, in which a terminal-spined egg was seen in an 'immature' female, and concludes that by this observation my theory 'is disposed of.' I cannot help finding that Dr. SAMBON is somewhat hasty in disposing of theories which are in contradiction with his own. I see that my young friend LEIPER states the immature condition of the specimen, but I do not see that he states the absence of spermatozoa in her oviduct. Was the worm, therefore, fertilized, or was it not? I further think that for everyone who will look at the case with an open mind it is clear that there is no mathematical line of demarcation between 'maturity' and 'immaturity.' The eggs are formed in the 'ootype' which is situated at about the middle of the body at the posterior end of the long uterus. In the young females found in the portal vein the eggs are lateral-spined. They are gradually pushed along the uterus till at the end they are

expelled by the genital aperture situated behind the ventral sucker. Other eggs may follow the first, but, according to our present knowledge, the number of those present at a time remains limited to 5 or 6. When the female is impregnated the formation of normal eggs begins. At about this period we ought to expect the uterus to show, in its hindmost part, a number of terminal-spined eggs, while the anterior part may still contain one or some lateral-spined ova. Such was the case in the specimen observed by BILHARZ; the analogy it presents to some of the Trematodes described above is complete. Had BILHARZ happened to see the specimen an hour or a day later the last lateral-spined egg would have been laid; the specimen would have been 'mature.' Had he happened to see it a day or a week earlier, no terminal-spined eggs might have been formed yet; the specimen was 'immature.' As he actually saw it it was half 'mature' and half 'immature.' For the moment, therefore, I see no reason why my theory should be annihilated by the one accidental observation of Dr. LEIPER,—admitting even that the egg in question were really terminal-spined, and did not only appear as such because the lateral spine was turned towards, or away from, the observer.

Dr. LEIPER himself says in the discussion (p. 45) that his observation makes him believe 'that the explanation was not correct which relied solely upon immaturity as the cause of the lateral spine.' This is quite right, but I have not pretended either, that immaturity is the sole cause of the lateral spine. Dr. SAMBON, in quoting my theory, makes me say that 'the eggs bearing a terminal (obviously a misprint for "lateral") spine probably represent the product of unfertilized females.' He thus does not notice that there is a slight but very important difference between saying 'lateral-spined eggs are the product of unfertilized females,' and saying, as I really have done, that 'unfertilized females are not capable of producing other than abnormal eggs.' As a matter of fact, several earlier authors have pointed out how fertilized females might, under certain conditions, produce lateral-spined eggs also. These suppositions have up to the present day not been proved as true, but they have not been disproved either, and it is at least not impossible that what those authors surmise may actually happen. At any rate, I have never pretended, and do not pretend, that immaturity is the sole cause of the lateral spine; nor is it impossible that immature females, although producing

as a rule, lateral-spined eggs, may not, as an exception, produce one or another terminal-spined egg. Biological processes can never be pressed into a mathematical formula to which there is no possible exception.

Speaking of the significance of these eggs I will provisionally quote the opinion of HOLCOMB, who says (1907, p. 62): 'The West Indian infection proves that the lateral-spined eggs are not the eggs of unfertilized females, and some of my cases, which were under observation for one year or more, show only too well the persistence of the type of egg cannot be attributed only to young females.' Before I can respond to this argument several other points must be discussed: I, therefore, at this place, limit myself to quoting HOLCOMB'S objection, and will return to the point later.

The habitat of the mature Bilharzia worms are the finer ramifications, in the first place, of the vesical and, in the second place, of the rectal veins. As a logical consequence of my theory, one ought to expect that, there, they produce terminal-spined eggs only. Observation shows that the lesions actually contain such eggs in enormous numbers, and very often absolutely alone. Some stray lateral-spined eggs found at the one place or the other would not shake this rule. Even when large numbers were found in numerous cases the fact would not prove any specific nature of these eggs unless it were shown, either that unimpregnated females cannot possibly get to the same places, or, that impregnated females cannot under any circumstances form lateral-spined eggs.

The young females living in the portal system produce lateral-spined ova, and successively expel them into the surrounding blood. Since the ova are not by themselves mobile the blood stream will carry them deeper into the liver, where, logically, they must finally become arrested in those vessels whose diameter equals their own. The theory held by me thus leads to the logical consequence that lateral-spined eggs must first and foremost accumulate in the liver. Their frequent and often plentiful occurrence in that organ is a well-known fact; indeed it represents another of the pillars on which my theory rests. According to the general belief (which I share) the worms grown up in the portal system reach their definite habitat in the pelvic organs by active wanderings, the vigorous males carrying the weaker females with them in the gynaecophoric canal. It is,

however, possible that the females are capable also of undertaking the wandering alone. As a matter of fact, isolated females have been seen in various veins; but it is not sure whether they got there alone or by the help of males whom they afterwards abandoned. During this journey all females go on laying eggs—at first abnormal ones, later (i.e., after they have become impregnated), normal ones. In all wider vessels, these eggs also are taken up by the blood stream and carried back to the liver where they join those which have arrived previously. There is, however, the probability that, now, terminal-spined ones may be among them; observation tells us that indeed these occur in the liver, though in numbers which vary considerably in the individual cases. But from what has been said above we may derive as the general rule that the lateral-spined eggs will prevail, the longer the females had to wait for fertilization, whereas the terminal-spined eggs will prevail, the sooner the females became fertilized.

In the walls of bladder and rectum the worms make their way into the finer ramifications the diameter of which gradually becomes equal or even less than that of the male. From this point onwards it is difficult for the eggs laid by the female to escape into the general circulation. Pictures I have seen in sections of the vesical and rectal wall even seem to indicate that the females can stretch their (already thin) bodies to such an extent, escaping at the same time more or less from the gynaecophoric canal of the male, that their heads (close to which the genital aperture is situated) reach very fine capillaries. Eggs deposited there—either singly or in groups—would be kept in place by the walls of the vessels closing in upon them as soon as the female withdraws to her original place. The process may be repeated more or less often, a whole area becoming thus stuffed with ova. I have not seen the process here described actually going on; it is also probable that many variations occur; but the chief details are based on observed facts.

The eggs, though originally deposited in the blood vessels, finally appear in the urine or the faeces: they must have passed through the tissues of the organs. I do not consider it as illogical to admit that what happens to the eggs in the walls of bladder and rectum may also happen to the eggs in the liver. Observation actually shows

them in the tissue of the organ: they, therefore, have left the blood vessels as they have in the pelvic organs. Admitting that they change their place in the tissue one will easily see that several things may happen. I will at this place only mention the possibility of some eggs getting into a blood vessel of the hepatic system. Should this take place the blood stream would carry them away from the liver; the next place where they are likely to become arrested again is the lung. Observation has shown that the organ in which, next to the liver, lateral-spined eggs are most commonly found is the lung. What happened in the liver may happen in the lung; the eggs escaping from the latter would be carried by the arterial circulation to every possible organ. As a matter of fact, stray lateral-spined eggs have been seen at very different places. I will add that the way just described is not the only one by which they may reach the lung and other organs; however, these details may here be omitted as having no direct bearing on the questions under discussion.

Returning to the eggs in the liver, there is, in addition to the possibility above mentioned, the other possibility of their getting into a part of the biliary system. In this case they would be carried to the gall bladder and thence into the intestine, from which they would be voided with the faeces. After I had ascertained that in certain cases lateral-spined eggs could not possibly be derived from rectal lesions, I came to think of this possibility as an explanation of their presence in the faeces. Observation showed that the theory held good in this case also. In the first body available the first preparation made of the bile from the bladder revealed under the microscope four lateral-spined eggs; others were found in scrapings of the bladder wall, in the bile duct and all along the intestine. Three other cases examined subsequently presented similar conditions; I have not deemed it necessary to examine more. The theory had led to a conclusion which when tested by observation proved to be correct.

This is one explanation I have to offer for the occurrence of the lateral-spined eggs in the faeces. There are others still, but I will not allow myself to enter upon details which have no direct bearing upon the question which here interests us. Observation has thus shown that the occurrence of lateral-spined eggs in the faeces of

living patients is not by any means a proof of the infection of the intestine proper, and quite especially of the rectum. These eggs may, and in many cases do, come from the liver; the only question of importance which remains to be answered in this connection is the question as to the reasons, why the infection of the liver is, in certain localities as the exception, in other localities as the rule, not followed by an infection of the bladder. I will show later that there is a possibility—and to my mind not even a very far-fetched possibility—to explain this curious difference, without the help of a mythical 'new species.'

We have seen above that the 'specific pathogenic action' assigned by Dr. SAMBON to his *Sch. mansoni* does not exist, for *Sch. hæmatobium* is capable of producing the same lesions. We see now that there is no 'special anatomical habitat' either, for the lateral-spined eggs appearing in the faeces of living persons may be such of young *Sch. hæmatobium* deposited in, and voided from, the liver. It may be added that up to the present nobody appears to have seen lateral-spined eggs in females imbedded in the gynaecophoric canal of the male, and the latter imbedded in a vein of the rectal wall. I have myself seen in situ quite a number of such females, but they only contained terminal-spined ova. I do not attribute any demonstrative value to these statements, but may point out that Dr. SAMBON's theory would find an important support if he, or somebody else, could produce females collected under the conditions above mentioned, which possessed in her uterus exclusively, and as many lateral-spined eggs as the ordinary females possess terminal-spined eggs under the same conditions. I have in some instances counted the eggs in females collected from the mesenterial, rectal, and vesical veins, and have found them to vary in number between 80 and 150.*

We now come to Dr. SAMBON's third proof, the 'peculiar geographical distribution' of *Sch. mansoni*. I may freely confess

* In a case quite recently examined I found a little colony of worms in the haemorrhoidal vein, about 7 cm. distant from the anus. There were five couples and two bachelor males. All males measure (after preservation) 5-6 mm. in length; their testicles do not yet contain free spermatozoa. The females average 7 mm. in length. Their internal genital organs do not show spermatozoa. Three are also entirely free from eggs; the two others contain each one lateral-spined ovum in the ootype, none in the uterus. This observation shows that the worms may leave the liver before sexual maturity is attained, but otherwise agrees with the theory. (Note added while reading the proofs.)

that when I first read the author's own statements the statistical observations indeed seemed to strongly favour his view. However, on testing the evidence somewhat more seriously, I find that matters change their aspect considerably. Unfortunately, a number of the papers on which the evidence is based are not actually within my reach, and cannot, therefore, be compared. From those I possess I see that Dr. SAMBON quotes the literature in a rather unusual manner.

Beginning with the West Indies it is true that according to the report by Dr. HOLCOMB (1907), rectal Bilharziosis is very common in those parts and vesical infection is rare. That the latter is actually absent cannot be said, for HOLCOMB enumerates four cases (one in a man from Guatemala, two in persons from Panama and one in a Porto Rican) in which the urine contained the terminal-spined ova, in one case even combined with the presence of lateral-spined ova in the faeces. Dr. SAMBON does not mention these cases, but only says that 'endemic haematuria' is not known in the West Indies. HOLCOMB states that he was not informed where, in the four cases, the infection was obtained. Since it should not have been difficult to find out whether the infected persons had been to Africa, one may I think reasonably assume that the infection was acquired in loco. At any rate, there is no proof that it was not of local origin. However, I will not place great weight on these cases, owing to the fact that the place of infection is, though fairly clear, yet not positively ascertained. I consider it as more important that Dr. HOLCOMB has recently himself observed a case of urinary bilharziosis (information by letter). The most important case is that contracted at Martinique and very carefully studied by LETULLE (1905). LETULLE did not yet know of *Sch. mansoni* and the specific pathogenic action attributed to it. But he emphatically states that he found the bladder entirely free from infection. In the intestinal lesions, the lateral-spined eggs of '*Sch. mansoni*' were seen in company with the terminal-spined eggs of *Sch. hæmatobium*. The dimensions of all of them, by the way, agree very well with those of *Sch. hæmatobium*, if one remembers the fact that the latter increase considerably in size during their embryonic development. Dr. SAMBON mentions LETULLE's case as one of 'MANSON's Bilharziosis' (p. 32), but he does not mention that in this case (it is, so

far as I am aware, the first case of 'MANSON's Bilharziosis' thoroughly studied from a pathological point of view) both forms of eggs were found.

Passing now to Africa, *Sch. hæmatobium* is, according to Dr. SAMBON, alone present in Cape Colony. He refers to HARLEY's observations and quotes from this author's article: 'In all my own cases I can positively say that only one form of egg has existed, namely, that with a terminal spine. Variation in the size, length, and outline of the egg is often observable, but I have never seen any egg with even a tendency to the formation of a side spine. I even doubt whether this peculiar form exists in the *Distomum hæmatobium* itself.' I have unfortunately no access to HARLEY's paper, but LEUCKHART also mentions it, and he says: 'Restricted exclusively to the possibility of examining the urine of his patients HARLEY had no knowledge of the existence of the eggs with lateral spines, and, therefore, considered the worms as a species different from that of Egypt' (p. 507). It is thus true that HARLEY observed terminal-spined eggs only, but, unless LEUCKART's remark is incorrect (for which assumption there is not the slighest reason), simply because he had no occasion to examine faeces in which the lateral spines are found. Dr. SAMBON then refers to the observations of BROCK, and quotes: 'that BROCK and others stated that they had never encountered the egg with the lateral spine.' But, here again, BROCK himself says (p. 6): 'I have only been able to study the ova as they appear in the urine of patients suffering from Bilharzia.' I will not ask how it is possible that Dr. SAMBON makes such misleading statements in an article which apparently claims to be taken seriously. For there is no doubt that the observations of HARLEY and BROCK are anything but demonstrative of the absence of lateral-spined eggs in South Africa, as Dr. SAMBON makes it appear by his quotations. As to the observations made in the Congo Free State, I am sorry that I have no access to the original article, and, therefore, cannot say how far its contents correspond to the summary given by SAMBON. In the discussion Dr. LOW states that in Uganda he saw exclusively rectal cases, but often also terminal-spined eggs 'in the rectum' (1907, p. 45).

Looking at this geographical evidence, as it now appears, the observer will first be struck by the fact that it has entirely lost its

original neatness. The statements, mostly based on clinical observations during the daily routine work, seem contradictory, and nowhere is there a sharp line of demarcation left. I, for one, cannot see any trace of a 'peculiar geographical distribution' of the two shapes of the egg which are said to be distinctive of the alleged two species—admitting even that the statistics are all equally reliable, i.e., made with special regard to the question at issue. But, be that as it may; there is certainly no doubt about the evidence supplied by the case of LETULLE. This was, according to Dr. SAMBON's own words, a case of 'MANSON's Bilharziosis,' and, so far as I can judge, one absolutely typical both as regards the origin (Martinique) and the clinical and pathological aspects. Nevertheless, a careful study of the lesions revealed the presence in them of both forms of eggs. After SAMBON, it would thus have been a 'combined' infection with *Sch. mansoni* and *Sch. hæmatobium*; an infection, however, in which *Sch. hæmatobium* did not produce its own lesions, but those of '*Sch. mansoni*.' It remains for Dr. SAMBON to show the way out of this labyrinth. For me there is no difficulty, for I say that both forms of eggs belong to the same species, and that the apparent differences between vesical and rectal Bilharziosis are not due to a difference in the species of the parasite, but to reasons which must be looked for elsewhere; we shall see later what they may be. If I were to make a 'prophecy' I would say that in almost all cases of 'MANSON's Bilharziosis,' if they are so thoroughly examined as the Martinique case was by LETULLE, the eggs of *Sch. hæmatobium* will be found among the eggs of '*Sch. mansoni*.' (I have just emphasized the word 'almost'; we shall see later that there are certain conditions under which the lateral-spined ought to be present quite alone). I especially recommend for examination the liver. It is a pity that it was not studied in LETULLE's case; but I can easily comprehend that there was for LETULLE no visible reason to look for ova there.

If we now compare the various pictures offered by Bilharziosis according to observers with those known from Egypt, there is no difference left except one, and this, as I must frankly confess, is a very striking one, namely, the apparent irregularity in the localisation of the lesions. In order to make my case complete I will try to show that the biology, such as I

interpret it, of *Sch. hæmatobium* is perfectly sufficient to throw light on this difference also.

Before advancing any further, and in order to avoid any misunderstandings, I will repeat that I do not ignore that what I have said with regard to the nature of the lateral-spined eggs, and what I am going to say hereafter with regard to the differences in the clinical aspect of the disease, is a 'theory,' inasmuch as it has not yet been established by experimental proof. In the absence of such proof, the only thing the scientific man, desirous of advancing our knowledge, can do, is to collect carefully as many isolated facts as may be obtainable; to separate those which are (presumably) essential from those which are (presumably) accidental, and to piece all of them together into a continuous train of events. This is what I have been endeavouring to do; I cannot imagine that a theory thus built up can be wrong in its fundamentals. It must, of course, be incomplete, or may be erroneous in details. I have already pointed out that Bilharziosis, in its varying aspects, presents a peculiarly complex problem, both as regards its pathogeny and the biology of the parasite. I do not think that I am wrong when I say that the latter represents the basis of the former, especially so far as the development and the behaviour of the worm within the human body are concerned. When, with regard to this part, I have knowledge of a good number of details, I owe that to the kind collaboration of my colleagues of the Medical School and the Kasr el Aini Hospital, Dr. ELLIOT SMITH, the Anatomist, Drs. SYMMERS (now of Queen's College, Belfast) and FERGUSON, Pathologists, Dr. MADDEN and Mr. FR. MILTON, Surgeons, who have discussed with me the observations they had occasion to make during their professional work, and have given me many a valuable hint as to details with which I am less familiar. A priori, the various observations might have been explained in various ways, but the right explanation could only be one which fitted in with the biology of the parasite. *Sch. hæmatobium* has thus far successfully resisted all attempts at revealing the secret of its development. Nevertheless, we know a number of facts which definitely settle certain details; as to others, all we can do at present is to accept what seems to be most probable. For me, everything is probable as soon as it has been demonstrated in the nearest natural relatives of the *Sch. hæmatobium*, i.e., either in other Schistosomes, or

in the digenetic Trematodes. I have acted according to this principle in formulating my theory. As a matter of course, in doing this, I depend upon our present knowledge. It often happens that a theory which seems probable and natural at one time is at another upset by new facts which, though not annihilating the older facts, yet make them appear in a different light. I cannot foretell at present what facts may be in store for us with regard to Bilharziosis, and, therefore, cannot say that the theory which I defend at present is the right one in every detail. But I think that I can claim that it is based upon a large number of anatomo-pathological and helminthological facts deliberately weighed and compared. I have thought these remarks necessary in the face of Dr. SAMBON's allusion that myself, and all the other workers in Egypt, have not been able within long years to find the solution of a problem which according to him was easy enough after all.

So as to be quite impersonal I will myself draw attention to an important biological point which I am not yet able to sufficiently account for. I am convinced that the lateral-spined eggs are abnormal and, probably, unfertilized. Nevertheless, when they appear in the faeces they very often contain a fully-developed miracidium. If we suppose, with some earlier authors, that mature females are, under certain circumstances, still capable of producing such lateral-spined eggs, the dilemma would resolve itself into the question as to what these conditions are (spontaneous contraction; pressure of the surrounding organs, either accidental or owing to their movements; &c.). I have already said that this point is not yet determined. To me it seems, on the whole, very little probable, that fully mature females continue to produce lateral-spined eggs. If this be true, the presence of the miracidia would forcibly indicate that the eggs are capable of developing by parthenogenesis. From what has been observed in the hermaphroditic Trematodes it appears that the eggs must be fertilized, in order to develop. A priori, one ought to expect the same also in the Trematodes with separate sexes. However, considering the unmistakable disadvantages connected with this separation in regard to the preservation of the race under the peculiar circumstances under which the Schistosomes are living, and considering further the very complicated development of many Trematodes, in which often several

asexual generations occur before the sexual stage is again reached, I would hesitate to pronounce a hasty conclusion. At any rate, the presence of fully developed miracidia in lateral-spined eggs is a point which still requires to be cleared up biologically. For the question as to the specific nature of the lateral-spined ovum the point is of no consequence; for its combined presence in the same individual with the terminal-spined egg is evidence enough that only individual conditions can be responsible for its formation.

I will now try to show that the strange and striking difference offered by the clinical and pathological pictures of Bilharziosis in various places is not incapable of explanation if we consider the presumptive life history of the parasite, in connection with the habits of the host and the conditions of the country. In order to make this clear I must start from the beginning.

The miracidium (often inappropriately called 'embryo') contains in its abdominal cavity the so-called 'germinal cells,' the significance and ultimate fate of which are well known from their comparative study in various other Trematodes. The existence of these cells in the Bilharzia miracidium is absolute evidence that the miracidium cannot develop directly into an adult worm, but must pass through the stage of the 'sporocyst' which, in its turn, produces, either (and probably) at once, or by one or more intermediate generations, the definite worms. All attempts made by former authors to discover an intermediary host in which this development is gone through, have failed, and so have my own efforts. I have examined hundreds of specimens of all the molluscs common in the Nile valley, without finding any sporocyst which might have been brought into relation with the Bilharzia worms. I have placed quantities of free swimming miracidia in contact with the same molluscs, without obtaining an infection. It is easy to infect molluscs with miracidia of species which actually develop in them. I will not enter into details, but only say that the Bilharzia miracidia were never seen to take any notice of any mollusc in their neighbourhood, whereas others developing in a certain mollusc soon begin to swarm about it, and may, under the microscope, even be observed to enter into it. The same negative results were obtained with larvae of insects, with fishes, and with plants. I am

thus forced to the conviction that Man himself acts as intermediary host.

If this conclusion is correct it leads to the important consequence that the spread of the *Sch. hæmatobium* is not limited by the natural geographical distribution of a special intermediary host. It can spread wherever man carries it, so long as, and in so far as, the climatic and hydrographic conditions are favourable for its development. With regard to this point, I entirely disagree with Sir PATRICK MANSON who says (1907, p. 653) that the peculiar geographical limitations of *Sch. hæmatobium* are difficult to explain if it does not require the services of an intermediary host. However, I also hold that *Sch. hæmatobium* is by no means geographically so limited as it appears to be to the defenders of the existence of *Sch. mansoni.*

No investigator has hitherto succeeded in keeping the miracidia alive for more than 30-40 hours; in my personal experiments, the upper limit found was 28 hours. They must find some new shelter within this time. If they are destined to return into man directly, two possibilities are, a priori, imaginable, viz., that they enter by the mouth, or that they enter by the skin. I have found by experiment that hydrochloric acid diluted with water to the extent of 1 : 2000 kills them within 2-3 minutes, a solution of 1 : 1000 almost instantaneously; by exclusion I am thus led to the view that they enter by the skin. There are some other facts which may be interpreted in favour of this view; but I will not mention them here. In Man, the miracidium must develop into a sporocyst which, either directly, or indirectly, generates the Bilharzia worms.

We have already seen that the only organ of the body thus far known to harbour young, and sometimes very young, worms is the liver. I therefrom conclude that the liver is the habitat of the sporocyst, from which the worms later escape into the portal vein. A priori, one might think of the possibility that they can escape also into the hepatic veins. As a matter of fact, they have been found comparatively often in the vena cava (KARTULIS), the lung (SYMMERS), &c. If the liver is the seat of the sporocyst it is a curious coincidence (perhaps it is not a mere coincidence) that, in the known intermediary hosts of other Trematodes, it is the liver which harbours the sporocysts.

At post mortems, it is not uncommon to find males alone in the portal vein. These males are often conspicuously of the same size, in other words, all of the same age. They must have been generated at about the same time; this would become comprehensible on the assumption that they are generated in one sporocyst. If one sporocyst may produce males I see no reason which forbids the assumption that the females take their origin in separate sporocysts. As females are, as a rule, found much more rarely than males, it may be admitted that male sporocysts are commoner than female.

This is the way in which, according to the facts at present available I am forced to explain the arrival of the parasites in the human body. I will now describe how I seem to see the connection between the special aspects of the disease and the habits of the population as they are observable, in the first place, in Egypt. In Egypt, Bilharziosis is very common. In the towns it is especially the children who are infected; among our students, there are always some who have, or have had, haematuria. Some of them assert emphatically that they have got it while in the country. In all of them the disease lasts for some years and then disappears. All severe cases come from the country. The Egyptian peasants usually work their fields in companies; sometimes of two or three, sometimes of several dozens; standing with their feet, and working with their hands, in the water or the mud. They often also bathe in companies in canals with slowly flowing water, pools, &c. One of them who is infected with urinary Bilharziosis, when urinating into the water, infects it with several hundreds, perhaps thousands, of eggs. In warm weather the miracidia hatch within a few minutes. They have at once the opportunity of finding a new shelter, either in the skin of the man who voided the eggs or in the legs or hands of one of his comrades working close by him. Many of the miracidia which enter the skin will not succeed in finding their way to the liver, but a few do so. These possibilities of infection are repeated every time a man urinates into the water. They are perhaps repeated every day the season of the Nile flood lasts. There is thus not only the possibility, but the extreme probability, that several miracidia attain their destination at short intervals.

The worms they give rise to in the liver are of about the same age. On this supposition, viz., that several miracidia succeed in

gaining the liver at short intervals, it becomes probable that, from the beginning, there will be males and females. In this case, the females, grown up to the sexual stage will not have to wait long for fertilization. They will produce a few abnormal eggs, but are soon taken up by the males and carried to the pelvic organs. On the whole, therefore, only a comparatively restricted number of lateral-spined eggs will be deposited in the liver; they may, subsequently, be joined by larger numbers of terminal-spined.

The chief habitat of the adult worms is undoubtedly the bladder. The chief vein which leads them there is the inferior mesenteric vein. I will point out in passing, that during the journey an occasional couple, before reaching the vein named, may accidentally get into a side branch coming from some other part of the bowel. In such a case the worms would give rise to an isolated focus of infection, or a separate growth at an unusual place. Such unusually located lesions have often been observed, and are, I think, correctly explained in the way alluded to. The inferior mesenteric vein leads the worms to the haemorrhoidal plexus close to the anus, but not immediately to the bladder. In order to settle the very important anatomical point whether there is a connection, wide enough to let the worms pass, between the veins of the rectum and those of the bladder, Dr. ELLIOT SMITH has very kindly made a dissection of the respective parts. Since he proposes to return to this anatomical question in detail himself, I here limit myself to the statement that such connections exist, wide enough to allow, not one couple only, but two and perhaps even three to pass side by side. I have subsequently found the worms in veins which, to judge from their width and course, were such connecting branches between rectum and bladder.

I will not omit in this connection to recall the remarkable degree in which Trematodes are able to contract their bodies. I have under the microscope followed Cercariae entering into tadpoles and insect larvae. The actual opening they make in the skin of these 'supplementary' hosts is often so small that the worms assume the shape of an hour glass; but they get through it, evidently without difficulty. There is, therefore, every probability that male Bilharzia worms may manage to travel through vessels the ordinary diameter of which does not exceed a half or even a third of a millimetre.

There is thus no doubt that the parasites have a direct route from

the portal vein to the bladder. Another very important question is why they do not remain in the veins of the rectum (or the intestine in general, which is apparently the original habitat of the Schistosomes), and how they find their way through the (comparatively) few connections between rectal and vesical veins into the latter. In order to explain this remarkable 'knowledge of anatomy' I will draw attention to some well-known facts derived from the comparative biology of other parasitic worms. The larvae of the *Filaria bancrofti*, e.g., after having been sucked up by a mosquito, leave the intestine by perforating its wall, and make their way into the thoracic muscles; the larvae of the *Filaria immitis* do the same, but seek the Malpighian tubes. The mature Ankylostoma worms do not live irregularly scattered throughout the small intestine, but chiefly accumulate in a certain region. Many Amphistomes inhabit the first stomach of their hosts (Ruminants), but the specimens found there are, according to my personal experience, never below a certain size. The young stages live, often by hundreds, in the small intestine. They have been swallowed along with the food, but do not at once settle in the stomach (which they have to traverse in order to get to the small intestine); it is not until they have reached a certain size at this provisional habitat in the small intestine that they return to the first stomach which is their definite habitat.

In all these cases the worms must be guided by something which makes them find their place of destination. I have no doubt that this something is given in the peculiar chemical composition of the organs, or the juices, at the respective places; in other words, the wanderings come under the phenomena of 'chemiotaxis.' One might suppose that the conditions in the small intestine of man are about the same throughout its total length (at least behind the entrance of the bile ducts). But the fact that the Ankylostoma worms normally settle in the anterior half only, is to my mind evidence that there must be differences which to the worms are noticeable, and lead them to select one special place in preference to any other. The fact that stray specimens may often be found more or less far away from this place, does not shake the rule; these specimens are 'the exceptions which strengthen the rule.'

Starting from these reflections I conclude that the Bilharzia worms, also, are guided in their journey by chemiotactic influences. I do

not think it unreasonable to conceive that the venous blood coming from the bladder is chemically slightly different from that of the rectal veins, and that this difference, slight as it may be, exercises an attractive influence on the worms, thus 'leading' them to the bladder. It is in this connection certainly not without significance that the whole journey goes against the blood stream, just as the dog scents the game against the wind, but not with the wind. At any rate, the veins of the bladder seem to be those first sought by the worms, although the rectal veins are nearer and would serve their purpose (to bring the eggs to some place where they can easily reach the outer world) equally well. As a matter of fact, the other *Schistosoma* species known are chiefly inhabitants of the intestinal veins. In *Sch. hæmatobium* the first infection of previously healthy persons seems to normally concern the bladder, whereas (apparently) the rectum becomes implicated after repeated infections only. One might almost imagine that after numerous eggs have been deposited in the bladder, and the normal function of the organ has become more or less impaired, the blood loses for the worms its peculiar 'scent.' There may also be mechanical reasons which keep them in the rectal veins in larger numbers than before, &c. In this, or some similar way, the rectum would gradually become infected after the bladder. However, I do not find any reasonable objection either, to the assumption that in some cases some couples of worms might from the beginning remain and establish themselves in the veins of the rectum. Owing to the kindness of Dr. Ferguson, I have recently had the opportunity of examining several cases of 'early Bilharziosis of the bladder,' in which the most scrupulous inspection of the rectum could not detect any visible change in the normal aspect of that organ. Nevertheless, quite a number of (terminal-spined) eggs were found in the residue after a part of the rectal wall had been macerated in caustic potash.

In bladder (or rectum) the real oviposition begins; the eggs are at first scarce, but gradually increase in number. They are laid in the blood vessels but afterwards escape into the tissues and are finally voided from the body after having traversed the mucous membrane of the bladder (or rectum). We do not yet know how long it takes them to accomplish this journey, but some will reach the end of it in a comparatively short time, whereas others may not

succeed even after a long time. In any case, they do not appear in the urine (or the faeces) at once. The beginning of the haematuria is quite insidious; from Egypt many cases are known where there was not even haematuria; the eggs were accidentally discovered in examinations of the urine made for other purposes.

While travelling through the tissues the eggs undergo their embryonic development. The eggs at any time visible in the uterus of the female worms invariably contain an undivided egg-cell. As the embryo develops within the egg shell, the egg itself increases in size; that with a mature miracidium inside measures, on an average, 0·13 to 0·15 by 0·04 to 0·06 mm., whereas immediately after formation in the ootype it is only 0·08 to 0·09 by 0·03 to 0·04 mm. in dimension. During the embryonic development, many embryos die. Their bodies become gradually decomposed, and afterwards replaced by calcareous masses; the eggs become 'calcified.' Their appearance in this state is known well enough, so I do not want to dwell on it. With the moment of the death of the embryo, the increase in size of the whole egg is stopped. The calcified eggs thus present the well-known variations in size; they remain small when they died early, they are larger when they died later.

Appearances I have seen in many sections of bilharzial tissues (of recent and old cases) make me believe that dead eggs are no longer capable of traversing the tissues as easily as living ones do. They will thus remain in the tissues more easily. In cases of very long standing they are often found quite alone; in other words, cases in which calcified eggs are found in the tissues, or voided with the excreta, are old cases.

The age the worms may reach is not yet known; for the sake of my argument we will assume that it be three years. Three years, therefore, after a man has become infected (and has had no occasion since to become re-infected!) adult worms will no longer be in his system. But during their three years' life the females have produced an immense quantity of eggs. Many of them have been voided during the same time, but as many, probably many more, are still in the tissues, and continue to be voided with the urine (or the faeces) for a more or less longer period. But their presence, and even the presence of a live miracidium in them, is by no means a sign that the worms which produced the eggs are still alive. I may mention in

this connection that practitioners have now and again tried to relieve their patients by administering drugs with the view of killing the adult worms. Considering what has just been said, and considering the other fact that the great majority of Bilharzia patients do not come for relief before the evil is more or less far advanced, it will at once be understood that in nine cases out of ten every attempt at the worms will be too late. That the eggs voided still harbour a live and active miracidium is no proof that they must be derived from a comparatively recent infection. We know of cases which, according to our present knowledge, cannot be explained unless by the assumption that within the human body the miracidia enclosed in their egg shells are capable of retaining their vitality for many years. The fact is by no means an uncommon one among the parasitic worms; the encapsuled Trichina or the wheat-eel dried up in its grain are well-known instances of longevity, which also show its biological significance. For it is not difficult to understand that the longer an immature parasite is able to wait for its chance, the greater becomes the probability that it obtains the chance for getting under those conditions which allow it to grow to sexual maturity and propagate its race. I think that the capability of the miracidia, in the eggs which are not at once voided from the body, to remain alive for a very long time must be looked at from this same point of view.

We have so far considered (theoretically) what I should like to call the normal course of events, i.e., that which takes place in localities where it is comparatively easy for the miracidia to find a new host within a short time. Under these conditions there is every probability that the females have not long to wait for the males. They produce few abnormal ova, the liver remains almost free, but terminal-spined ova are deposited plentifully in the bladder or rectum, as the case may be. There is 'urinary Bilharziosis' characterised by the apparition of terminal-spined eggs in the urine; the same eggs may also appear in the faeces, but lateral-spined ones will be so scarce that they seem to be altogether absent. We will now consider what is likely to happen under conditions which are no longer so favourable to the worms.

A man, for instance, does not work, or bathe, in the water day by day, but only at intervals of weeks or months; he does not remain in the water long, but for some minutes or an hour only; he avoids

the close company of others; the water itself may perhaps be quickly flowing, thus sweeping the miracidia away from the place where they have been hatched, &c., &c. Under all these conditions, combined with the short time the miracidia are able to remain alive in water, the chances of entering the skin of a new human host are considerably reduced for any which may be in the water. Many a time not one will succeed in finding him and entering his body. On a single occasion, however, a few miracidia manage to enter his skin, and one gets safely to the liver. It produces males (we know that these are much commoner than the females; the probability of picking up a male sporocyst is therefore greater). The worms grow to sexual maturity, but finding no females they wait perhaps for a certain time, and then undertake the journey to the pelvic organs alone. After some time, the liver is again free from worms; the infection, although it has taken place, remains without consequences.

The man continues exposing himself to the conditions for infection as indicated. What happened previously may be repeated at intervals, but on one of these occasions a miracidium may enter his body which produces female worms. These in due time begin to lay lateral-spined eggs. The oviposition goes on, perhaps, for a long time. The number of lateral-spined eggs increases steadily; all are carried to the liver. It is possible (I might say probable) that some of the females try to undertake their journey alone, but owing to their inferior muscular strength they may sooner or later be driven into some smaller side branch of the mesenterial, chiefly of the splenic and inferior mesenteric veins. Not one succeeds in making the entire long journey to the rectum and the bladder. Of the specimens that have left the chief track leading to these organs, one or the other may reach the wall of the large bowel, filling a small area with her lateral-spined eggs. At the end there will be a comparatively strong infection of the liver, and perhaps some isolated infected patches in the wall of the intestine, but no terminal-spined ova will ever appear, nor will there be a regular infection of the bladder. After some time the lateral-spined eggs of the liver begin to appear in the faeces, and they continue being voided in this way for several years. I have further above (p. 174) hinted at the possible existence of cases in which even the most careful post mortem examination would not detect any terminal-spined ovum in any organ; we here have the

conditions under which such cases must arise. This seems to me a suitable place, too, for inserting a biological remark. I have spoken above of the presence of fully developed miricidia in lateral-spined eggs. If these eggs are unfertilized, as I hold they are, they must be capable of developing by parthenogenesis. We now see the vital advantage the parasites would derive from such a capability for the propagation of their race in localities where the conditions for infection are scarce. I may confess that from this point of view the hypothesis loses for me much of its original strangeness.

I am now able to answer the objection raised against my interpretation of the lateral-spined eggs by HOLCOMB. I suppose that the author means to say in his argument that the lateral-spined eggs could not have been derived from young females because in the course of the year during which the eggs were observed the females, though young perhaps initially, ought to have grown to sexual maturity and thus passed on to the formation of terminal-spined eggs; in other words, during a year the eggs appearing in the faeces ought to have changed from the lateral-spined to the terminal-spined type. This objection of HOLCOMB would indeed hold good if the Bilharzia eggs, like those of the intestinal parasites, were voided from the body of the host within 24 or 48 hours after their oviposition. But we know that they come from the liver, in which they have been laid by young or unfertilized females within a comparatively short period, but from which they are voided as gradually as the terminal-spined eggs are from the bladder—quite irrespective of what has in the meantime become of the worms which laid them. The observation referred to by HOLCOMB is therefore no proof against my interpretation that the lateral-spined eggs are the products of young, or unfertilized, females. It is, on the contrary, an argument in favour of it, whereas it would have been a certain evidence against my views had HOLCOMB observed that the eggs changed their shape in a marked degree in so short a period.

If we admit attempts at independent wanderings on the part of the females, it may happen that some of them, succeeding in getting near the haemorrhoidal plexus of the rectum, may find there some males, remnants of a previous infection. Or else the host may contract a new infection with another male sporocyst while some females of a previous infection are still alive in the liver. In both cases matters

would assume what we have called above their normal course. After due time terminal-spined ova would appear in the urine, while the lateral-spined eggs of the liver continue being voided by way of the rectum. I will not spin out this narrative any more. I think it will now be seen that in the way alluded to the clinical and pathological picture shown by the infection in any particular individual must depend upon the more or less favourable nature of the conditions of infection to which the individual has been exposed. A first infection with one or some male sporocysts would not lead to any consequence. A first infection with a female sporocyst would give a picture typical of 'MANSON's Bilharziosis,' i.e., an untouched bladder but lateral-spined eggs appearing for years in the faeces. In all countries where infection with *Sch. hæmatobium* is possible, a man once infected will, as a rule, be subject to the opportunity of re-infection. The aspect the disease will then show must vary with the intervals at which the infection becomes repeated, and with the sex of the worms which are acquired. We may get pictures such as represented by the case of LETULLE, where the external aspect still preserved the features of 'MANSON's Bilharziosis,' but where, internally, the normal, terminal-spined eggs were found in company with the lateral-spined type. If, finally, a man once only in his life, and perhaps for a few hours only, happens to come under a peculiar combination of circumstances which favour a simultaneous entrance of a larger number of miricidia, even in a country where otherwise the conditions for infection are unfavourable, he will contract for perhaps three, perhaps six, perhaps more years 'urinary Bilharziosis,' either pure, when he was not previously infected, or mixed with 'MANSON's Bilharziosis,' when he was infected with this peculiar type before. I am personally perfectly sure that the four cases of urinary Bilharziosis quoted by HOLCOMB were contracted in loco after the fashion here described.

On the whole, therefore, I do not, from my point of view, see any sharp line of demarcation between the two types. They are simply the opposite ends of a continuous series of intermediary stages.

I cannot quit this subject without drawing attention to another point which seems to me of great interest. We have seen that LETULLE expressly states that in his case the bladder was entirely free from infection. I can only interpret this statement in the sense that no pathological changes were perceivable in the bladder, but I

cannot quite believe that a close examination would not for all that have resulted in the detection of some eggs in the tissue of the bladder. It would have been of the utmost interest to know of what type they were. Cases like LETULLE's are rare in Egypt, because of the specially favourable conditions for infection in this country. But other observers may have an easier opportunity of examining them. Instead of a tedious preparation of sections, it would suffice to macerate a piece of the bladder-wall in caustic potash, and examine the residue. The shells of the Bilharzia eggs are not at once dissolved by this reagent, and even a few eggs would be found without difficulty, if present.

The conditions unfavourable for infection as they were suggested above will in general obtain in countries where there is a thin population, where the people do not come regularly in contact with water every day for a long part of the year, where they are not in the habit of working (or bathing) in companies, where there is not much water, or where the water, though abundant, flows quickly, &c., &c. I know neither the country nor the habits of the population in South Africa, in Uganda, in the Congo Free State, or in the West Indies, but I am fairly sure that on close observation of details the special aspect of the disease will be found to vary in accordance with the conditions for infection as they have been specified above.

I have previously alluded to the relative value of statistical observations. I can now illustrate what has been said there by an instructive example. HOLCOMB (who believes in '*Sch. mansoni*') after having given an extensive account of the cases of intestinal Bilharziosis observed in tropical America, compares them with the statistics published by various observers on the relative frequency of the different forms of Egyptian Bilharziosis (1907, p. 59). The main result he comes to is that, because there is in Cairo on an average one case of intestinal to 17 cases of vesical Bilharziosis, one ought to expect the same for the West Indies, if the cause of the disease were the same parasite in both countries. The actual observations, however, show the contrary: the intestinal form is very common and the vesical form is extremely rare; therefore, it appears that in the West Indies there is also another species of parasite.

For me, the same statistics do not prove anything beyond the bare fact that the disease shows in the two countries a remarkably

different aspect. In order to find out the reason for this fact, I would deem it necessary to analyse, if possible, all the factors that have, or may have, a share in bringing about the fact. The species of the parasite is one of them; the species of the host is another. But there are others, and amongst these the local conditions for infection are to my mind a factor of the first practical importance. This factor has been completely overlooked by HOLCOMB when he drew his conclusions, although its value is, so far as I have a judgment, fully recognised in modern epidemiology. Let us only assume it were possible to take one of the West Indian Islands, make its climatical and hydrographical conditions absolutely like those of Egypt, make the population (which is slightly infected with intestinal Bilharziosis) as dense as it is in the Nile Valley, make it live and work after the fashion of the Egyptian fellah, and then shut the Island off entirely from communication with the rest of the world; I have little doubt what the statistics would say some ten or twenty years hence.

Resuming now what has been said in this lengthy discussion, I must state that, of the evidence brought forward by Dr. SAMBON in order to justify the creation of '*Sch. mansoni*': 1, the zoological proof is absolutely insufficient; 2, the anatomo-pathological proof does not stand any serious test; and 3, the geographical proof is based upon a peculiarly one-sided interpretation of the literature. In all the evidence there is not the slightest detail which would really point to the existence of a distinct species in the West Indies and certain parts of Africa. It would be unwise on my part to go so far as to contend that such a species, or perhaps even several species, can not, altogether, exist. This is quite possible from the zoological point of view; but, zoologically, there is no possible doubt either that this species, or these species, must produce the same two shapes of eggs as does the *Sch. hæmatobium*, or else our present information is wholly incorrect. If, therefore, Dr. SAMBON wishes to maintain that there is an independent '*Sch. mansoni*' in the countries above-mentioned, the entire proof of its existence still remains to be given.

I cannot conclude this article without making some remarks of remonstrance with regard to another passage in Dr. SAMBON'S paper 'On the Part played by Metazoan Parasites in Tropical Pathology.' Speaking of the infection with *Agchylostoma duodenale*, he says that

the theory of infection by the skin 'now stands on the firm foundation of experimental proof.' But the printed abstract (Journ. Trop. Med. 1908, p. 34) then goes on to say that 'Dr. SAMBON doubted, however, whether the trachea-oesophagus part of the journey was more than conjecture; he thought the larvae could certainly reach the intestine by a safer and more direct route.' The author then refers to the larval stages of certain Cylicostomes found encysted in the intestinal wall, and to the larvae of *Sclerostomum vulgare* living in aneurysms of the mesenteric arteries, in horses. I presume that Dr. SAMBON means to indicate by this reference that there is a connection, or an analogy, between the development of the forms mentioned and that of the Ankylostomes. If my presumption is correct, I may say in answer that thoughts of this sort are as old as they are unfounded. They will be discussed in detail in the second part of my monograph on Ankylostoma which I am at present writing. If Dr. SAMBON further 'thinks' that the larvae could 'certainly' reach the intestine by a safer and more direct route, and if he 'doubts' whether the trachea-oesophagus part of the journey is 'more than conjecture,' I cannot help it. I will only state the following facts. My actual observations concerning the wanderings of the larvae were first made known in a paper read before the Sixth International Zoological Congress at Berne in 1904. They are printed in the 'Comptes rendus' of this Congress (1905a, p. 225f.), and again described in connection with some other questions concerning Ankylostomiasis in a later paper of mine (1905b). In Berne I exhibited a series of microscopical sections showing the larvae in the different stages of their journey. These preparations afterwards went for some time to Dr. OLIVER, of Newcastle-on-Tyne, who, with my authorisation, had lantern slides of some of them made which he used in connection with a paper read by him before the North of England Institute of Mining and Mechanical Engineers (1904). Some of these photographs were afterwards (I regret to say, without my authorisation) published in SIR PATRICK MANSON'S 'Lectures on Tropical Diseases' (1905); there is one (on page 20) showing larvae in a bronchus, and another showing a larva in the larynx (on page 21; it is erroneously labelled: 'in stomach'). In 1906 I had the pleasure of presenting to the London School of Tropical Medicine a series of preparations, accompanied by a detailed description, of all the important stages of the journey of the

larvae from the skin to the larynx. The tedious work of making these preparations was undertaken with the special purpose of sending them away in order to allow authors to form an individual opinion without great personal trouble, except, of course, that of looking at the preparations. Dr. SAMBON has not looked at them, nor has he consulted the literature before 'formulating his ideas.' I am sure that I do not under-estimate 'The Importance of Rational Inductive Methods in advancing Knowledge' (Journ. Trop. Med. 1908, p. 41), but I doubt whether 'ideas' like these (and several others formulated by Dr. SAMBON with regard to helminthological questions) have a right to be classed under that heading.

CAIRO, 16*th March*, 1908.

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THE PREVENTION OF DENGUE FEVER

BY

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Egypt has always been subject to periodical epidemics of dengue or dandy fever. In some of the towns the disease seems to be endemic, but sudden outbursts occur which spread all over the country. The disease presents the same characteristics as in other parts of the world and rarely gives rise to much difficulty in diagnosis. During epidemics the classical symptoms are very evident, including the pains, the apyretic period, and the rashes, which are sufficient to differentiate it from influenza. When pandemics of the disease occur in Egypt every town is invariably attacked, and few people escape. The death-rate, however, is very small as noticed elsewhere, though the debility and cardiac depression following an attack occasionally account for the sudden deaths of a few individuals who before were healthy. Since the discovery of the means of the transmission of malarial fevers it has been suggested by various writers that dengue fever is also conveyed from the sick to the healthy by the mosquito. Apparently Graham, of Beyrout, was the first to bring forward strong evidence of this,[1] and he named *Culex fatigans* Wied: as the culprit. Since that date further and conclusive evidence has been brought forward to support this statement.[2]

Dengue fever used to be very prevalent in Port Said, as in other parts of Egypt, up to the year 1905. An epidemic of the disease occurred in the town during the summer of 1904, and in the spring of 1905. This epidemic was part of an infection of all the towns of Egypt, and was most severe. The hospitals were full of cases, and patients actually contracted the disease in them. In Port Said almost everyone suffered from an attack, and the place was regarded

as fever-stricken and unhealthy. The town was full of mosquitoes, including two species of Anophelines, *Culex fatigans* and *Stegomyia* spp., in abundance. These mosquitoes were breeding in cess-pools under the houses, in basement cellars flooded with sewage, garden fountains, barrels containing water, and were a veritable pest day and night, summer and winter.

In May, 1906, a campaign against mosquitoes was instituted in the town as a general sanitary measure, with funds subscribed by the Egyptian Government and the Suez Canal Company, the support of Prince d'Arenberg, President of the Canal Company, and Sir Horace Pinching, late Director-General of the Egyptian Public Health Department, having been obtained. Two mosquito brigades were formed, one for the European and one for the native quarters of the town, and the oiling of all stagnant water practised once every week. Cess-pools were re-built and cellars filled up, with the result that within three months the mosquitoes were reduced to a negligible quantity, and mosquito nets largely dispensed with. Now, after two years, mosquitoes have become so rare in the town that they can be ignored, and malaria, though never very prevalent, has completely disappeared. But dengue fever has disappeared also, no case having been treated in Port Said since July, 1906. During the early part of that year, before the mosquito work began, dengue fever made its appearance as usual. Thirteen cases were treated in the hospital alone during April and May, and then as the mosquitoes disappeared the disease stopped and has not recurred since. In September, 1906, a severe epidemic raged throughout Egypt, beginning at Assouan and running rife in Cairo and Alexandria. It appeared in all the other towns, but Port Said and Ismailia remained free from it, no case occurring in either place. During the autumn of 1907 it again passed through Cairo and other parts of Egypt, but again Ismailia and Port Said escaped. Formerly the wards of the hospital in this town were full of cases of 'fever' during the summer months, but now the beds are used for other cases, which no longer contract fever although the mosquito nets have been removed. The extinction of the mosquito is greatly simplified in Egyptian towns owing to the dry summers, and the results can be easily watched. Port Said has a population of 56,000, and Ismailia 10,000. The cost of the mosquito work in the former is sixpence per head of population per year, while in the latter

town it is nearly eighteenpence per head, owing to the extensive irrigation works which have to be regularly dealt with.

It would seem, then, that the extermination of the domestic mosquito means the prevention of dengue fever, which, although not a very fatal disease, is one which causes endless misery in warm climates, and is as well a great hindrance to trade.

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THE LIFE HISTORY OF *TRYPANOSOMA LEWISI*

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The Trypanosomes, so far as at present known, are universally parasitic organisms inhabiting the blood and the body fluids of a variety of animals. In certain cases, the presence of trypanosomes produces the most marked pathological results. In others, the parasites are apparently quite harmless. Moreover, the same trypanosomes which are pathogenetic with respect to one animal, are often non-pathogenetic in the case of others. There is a tendency at present to attempt to draw a distinction in a classificatory sense between the so-called pathogenetic, and non-pathogenetic forms of trypanosomes. But even from the facts just referred to, it would seem to be clear that any such method of grouping can have but little real significance, and is more likely to entirely mislead enquiry than to throw any fresh light upon the singular and, morphologically speaking, closely knit group of organisms which the trypanosomes undoubtedly constitute.

There appear, as a matter of fact, to be two main groups of problems connected with the trypanosomes at the present time. The first is constituted by our ignorance of the complete features of the developmental cycle of even any well-known and characteristic representatives of the group. The second by the present impossibility of determining to what group of non-parasitic

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There appear, as a matter of fact, to be two main groups of problems connected with the trypanosomes at the present time. The first is constituted by our ignorance of the complete features of the developmental cycle of even any well-known and characteristic representatives of the group. The second by the present impossibility of determining to what group of non-parasitic

protozoa or protophytes the trypanosomes belong. There seems, however, to be little doubt that much of our present ignorance and confusion concerning the morphology and the life cycle of the trypanosomes has been due largely to an accident of technique, due in fact to the circumstance that the presence of trypanosomes can very readily be demonstrated by drying and staining the blood in which they are contained. It has so happened that by this method in its various forms, not only is the presence of trypanosomes demonstrated, but the preparations produced in this way are often extremely sharp, and beautiful to look at. It has consequently been only after a prolonged investigation of the effects produced upon such organisms, and on other forms of cells, by the process of drying, and by a careful comparison of the results obtained by this and other methods of fixation, that it has begun to be realised that dried preparations of cells, except for the purposes of demonstrating the presence of parasites, are generally as misleading as they are beautiful.

We have referred to this matter in our former papers, and we may say that all our further acquaintance with trypanosome morphology indicates clearly that the process of drying is entirely destructive of the finer cytological details, and consequently that it is altogether inapplicable to investigations wherein a true conception of the normal features of trypanosomes, or indeed of any cells, is necessary. In consequence of these considerations, we have entirely abandoned the use of dried preparations, and have relied here, as in our former work, upon modifications of the various methods of fixation in common use among Cytologists, together with such modifications of the various staining methods as have been found necessary during the course of the work. The features of the developmental cycle or life history of *T. lewisi*, although remarkable, in reality only assume their true proportion when considered in conjunction with the facts relating to the development of other trypanosomes that have now been studied.

For the sake of convenience, and for purposes of reference in subsequent portions of the present work, we shall in the first place briefly recapitulate our observations upon the developmental cycle in the case of *T. gambiense* and *T. equiperdum*. Before doing so, however, it is desirable to indicate the objects which we originally had in view in selecting the three forms, *T. gambiense*, *T. equiperdum*

and *T. lewisi.* Our primary intention with regard to *T. gambiense* was to ascertain what morphological results could be obtained with this form through the application of ordinary cytological methods in place of the usual drying process. But the subsequent results of this investigation were to reveal the existence of a life cycle among the parasites in the blood, which is definitely related to the alternating phases of presence and absence of trypanosomes in the circulation of infected rats.* The appearance of a cyclical metamorphosis among the trypanosomes in the blood indicated that the general conception of a special phase of their life history being definitely related to transference to another host (as is the case according to Schaudin with *T. noctuae*) might not be correct. Since, however, it is known that *T. gambiense* can be transmitted by the bites of tsetse flies, it was obvious that apart from investigations upon the transmitting insects, no ultimate conclusions could be arrived at with regard to this matter in the case of *T. gambiense.*

There existed, however, in the disease Dourine, a trypanosome which under normal and natural conditions is not transmitted by any fly, or biting animal, but simply through contact. It was clear, therefore, that in this instance we had a trypanosome life-history which was not normally complicated by the passage of the parasite through any intermediate host. Whatever life cycle *T. equiperdum* may possess, this cycle must be completed, and can be studied in the body of a single host. The acquisition of a knowledge of the facts relating to the life history of *T. equiperdum,* the parasite of Dourine, was therefore of the first importance as a means of affording comparative material during a consideration of the significance of the features of the life cycle of *T. gambiense* in the blood.

These two series of investigations in the case of *T. gambiense* and *T. equiperdum* having been undertaken, and both being related to parasites which produce violent and fatal maladies, it seemed further desirable to extend the investigation to some particular trypanosome which under normal circumstances belonged to the so-called non-pathogenetic forms. For this reason we selected *T. lewisi.* There was, however, another important point to be considered. *T. lewisi* can be transmitted from infected to non-

* Salvin-Moore and Breinl. Annals of Trop. Med. & Parasitology, Vol. I, No. 3, 1907.
" " Lancet, May 4, 1907.

infected rats by means of the rat louse, and consequently we had in this form of trypanosome a convenient object for investigating the changes which might take place among the trypanosomes when in the body of the louse, which here forms an intermediate host.

Passing now to the features of the developmental cycle in the parasites *T. gambiense* and *T. lewisi* in the blood, we find in the case of *T. gambiense** injected into rats that the disease is marked by alternating phases of presence and absence of parasites in the peripheral circulation. If numerous preparations be made of the blood at short intervals during the whole course of the infection, it is found that at the time the parasites are increasing in number in the blood, rapid multiplication is going forward by means of longitudinal fission. Such fission is accompanied by amitotic division of the nucleus and the intra-nuclear centrosome (nucleolus, karyosome), as well as by amitotic division of the extra-nuclear centrosome (blepharoplast), and lastly by the development of a new flagellum, and the final splitting of the original trypanosome into two separate flagellated cells, each containing a nucleus, an intra-nuclear centrosome, an extra-nuclear centrosome, and a flagellum.

Apart from the form of multiplication to which we have referred, no other form of reproduction takes place during the increase in the number of the parasites in the blood, and when we reach a point at or near the maximum number of trypanosomes in the circulation, the parasites cease to divide. At such periods it is found that in large numbers of them a stainable band develops from the extra-nuclear centrosome. This band extends, and finally becomes connected with the nucleus. It then breaks up and disappears. Subsequent to the development of the band, whether the trypanosomes again divide longitudinally, as in the case of *T. lewisi*, has not been ascertained. As we pass to those parts of the infection where the number of the parasites in the blood is falling, it is found that further rapid changes are taking place among the trypanosomes. The nuclei become more compact, vesicles appear in relation to them, and the nuclei, together with the vesicles, become separated from the outer portion of the cell, and enclosed by a delicate layer of cytoplasm. The remainder of such cells now disintegrates, and the composite body consisting of the nucleus, the vesicle, and a covering of

* Salvin-Moore and Breinl, *loc. cit.*

cytoplasm, becomes set free in the blood. These '*latent bodies*,' as we have termed them, are eventually carried out of the peripheral circulation, and are subsequently to be found in large numbers in the spleen, the bone-marrow, and other organs. The process just described goes on until there may be no parasites to be found at all in the peripheral blood, but the latent bodies do not disappear, and after a time some of them grow larger in size, develop a new extra-nuclear centrosome (apparently from the division of the intra-nuclear centrosome), become flagellated, and finally gradually transform themselves into trypanosomes again. When this process has been completed, a similar cycle is passed through in relation to each alternating period of presence, and absence of the parasites in the blood, which the infected animal may present. It should be noted, however, that the cycle does not necessarily go forward at the same rate in all the trypanosomes present in an infected rat. All through the alternating periods there may be found a few trypanosomes at almost every stage of the cycle.

Turning now to the development of *T. equiperdum*,* it is found that in horses the infection presents the same sort of alternation of presence and absence of parasites in the blood that occurs during the infection of rats with *T. gambiense*, but in relation to the study of *T. equiperdum* in horses a difficulty presents itself. In such infections the parasites are so few, that it is practically impossible to obtain a sufficient number of them at the various periods of the curve of the infection for any adequate study.

The same is the case when rabbits are infected with Dourine. We have therefore utilised rats, wherein the parasites multiply very rapidly, the features of the disease being as follows:—

After injection no parasites appear until about the third day. They then multiply with extreme rapidity, and kill the animal in about four days after their first appearance in the blood. In rats, therefore, there is during an infection of Dourine only one developmental period, which is completed at, or about, the time of death of the infected animal. From the time of their first appearance the parasites multiply by longitudinal division; the features of this process being the same as those occurring during the multiplication

* Salvin-Moore & Breinl. On the Life History of *Trypanosoma equiperdum*. Proc. Roy. Soc., 1908, Vol. 80.

of the trypanosomes with an infection of *T. gambiense.* After the multiplication has proceeded for some time, two normal changes occur. The first is constituted by the budding off of a mass from the nucleus, the mass eventually disappearing altogether. The second obviously corresponds to the formation of the stainable band at a similar period of an infection with *T. gambiense.* In the case of *T. equiperdum,* this process proceeds by the production of a large bud, originating from the extra-nuclear centrosome. The bud rapidly increases in size, becomes detached, and passes towards the nucleus, with which it finally becomes definitely associated. Afterwards the trypanosomes again pass through divisions, and subsequently enter upon another change. They become altered in shape. The extra-nuclear centrosome becomes related to a long neck of protoplasm. A vesicle appears in relation to the nucleus. The extra-nuclear centrosome, together with the protoplasmic neck and the flagellum, becomes detached, and a large round body remains wherein a new extra-nuclear centrosome is apparent. From this a fresh and exceedingly delicate flagellum grows out. The extra-nuclear centrosome divides, and a second flagellum is produced. These large round double flagellated forms obviously correspond to the latent bodies of *T. gambiense,* but owing to the fact that the disease invariably kills the rats at or about this period, we have as yet been unable to follow completely the transformation of the large latent bodies into trypanosomes once more. The latent bodies seem, however, in the first place to divide up and to produce smaller forms, which latter probably correspond to those occurring in the life cycle of *T. lewisi,* as we shall see.

T. lewisi is frequently found in the blood of wild rats in all parts of the world, but is rarely found in the blood of tame white rats. It is usually non-pathogenetic, and is relatively a large parasite. The morphology of the organism, and the various forms which it assumes in the blood, have been studied by several authors independently during recent years.* When a rat has become infected with *T. lewisi,*

* Laveran and Mesnil. Ann. de l'Institut Pasteur, Vol. XV, No. 9, p. 673.
Rabinowitsch and Kempner. Zeitsch. für Hygiene und Infectionskrankheiten, Vol. XXX, p. 251.
Wasielewski and Senn. Zeitsch. für Hygiene und Infectionskrankheiten, Vol. XXXIII, p. 246.
Ward, J. MacNeal. Life History of *Trypanosoma lewisi* and *Trypanosoma brucei.* Journ. Infect. Diseases, Vol. I, No. 4.
Prowazek. Studien über Säugethiertrypanosomen. Arb. a. d. Kaiserl, Gesundheitsamte, Vol. XXII, No. 2.

the parasites may be found in the blood in various forms at all periods of infection. Their development does not appear to occur in successive phases related to alternating presence and absence of trypanosomes in the blood, as is the case with *T. gambiense* and *T. equiperdum.* The elucidation of the developmental relationship of the various forms which thus exist together in the blood would at first sight seem to present a certain amount of difficulty, but in reality this difficulty is not so great as it appears. Thus the various authors who have already considered the subject are fairly well agreed with regard to the relationship in a developmental sense of the various forms one with another. Medium-sized parasites, such as those represented in fig. 1, certainly give rise by growth to the large pointed types represented in figs. 11-18, so also these latter unquestionably pass into the still larger round and regular multi-nucleated masses, such as those represented in fig. 23. Such masses may again in turn be found in all stages of breaking up into smaller bodies, and this process of dissociation certainly produces the characteristic rosettes, and other forms of temporary association commonly met with (figs. 26-30). Longitudinal division among the medium-sized forms such as those represented in fig. 1, appears to be a rare occurrence, but that it does take place is indicated by such types as those represented in figs. 2-5, wherein the nucleus or the extra-nuclear centrosome, or both, have become divided, and a second flagellum has arisen. We have, however, ourselves not encountered the late phases of division in such forms of *T. lewisi*, and have consequently only been able to figure the early stages of the process. The forms such as those represented in fig. 1 appear to certainly arise from, and to merge into, forms such as those represented in figs. 37-38, and these undoubtedly in turn have arisen from the products of the dissociation of the large multi-nucleated masses. Thus we appear to have a cycle of development in the blood, which, starting from any particular type such as those represented in fig. 1, passes through the phases represented in figs. 12-19 into the large forms represented in figs. 24-25. From this stage the cycle continues through stages such as those represented in figs. 30-38, and finally through growth and division the individual derivatives of the multi-nucleated masses pass back to the formation of types such as those originally chosen, as the starting point, and represented in fig. 1.

That such a progressive development in the case of each individual really represents the course of the cycle in the blood, receives complete confirmation from the study of the various morphological changes which take place at the successive periods of the cycle; for these changes, as we shall see, correspond closely to the analogous changes which occur during the development of *T. equiperdum* and *T. gambiense*, that is to say, in forms where the successive stages are passed through approximately simultaneously by the majority of the parasites during the course of infection.

The study of the morphology of *T. lewisi* may perhaps most simply be illustrated by taking in the first place examples such as those represented in fig. 1. In this condition the cell is long and pointed at both ends. The extra-nuclear centrosome, which is large, lies at a considerable distance from the pointed extremity of the cell. The extra-nuclear centrosome stains very deeply with many forms of coloration, and can be seen during life as a highly refractive body. In various stained preparations the extra-nuclear centrosome appears to be always related to a vacuole, or space in the surrounding cytoplasm, and the flagellum may present various appearances in relation both to the vacuole and the extra-nuclear centrosome. The flagellum, which is a long stainable band, extends in a curved course over the whole length of the body, and projects at the opposite end as a whip-lash. It is enclosed in a thin expansion of the cytoplasm, forming the so-called undulating membrane. The flagellum generally ends in a small body, or bead, near the extra-nuclear centrosome (fig. 1), but this is not always the case, for at times it certainly appears to run directly on to the extra-nuclear centrosome. When the flagellum is detached from the latter body, there can frequently be seen passing from the bead or thickening at the end of the flagellum, fine unstained strands which connect the bead with the extra-nuclear centrosome. The bead upon the end of the flagellum corresponds closely in appearance to the similar beads which are often found at the ends of the flagella among metazoan gametes, and such beads are in like manner often connected with the centrosomes by fine slightly staining strands. It would thus appear that so far as these structures among the trypanosomes can be directly homologised, the flagellum and its end-bead, together with the extra-nuclear centrosome, would correspond to the flagellum, bead, and centrosomes of many forms of

metazoan gametes. For this reason and others, to which we shall refer subsequently, we regard it as extremely misleading to name the end-bead a blepharoplast, and the extra-nuclear centrosome, a kineto-nucleus, for the end-bead (blepharoplast) does not present the relationships of a centrosome, or blepharoplast; while the extra-nuclear centrosome (kineto-nucleus) does so. Moreover, the extra-nuclear centrosome does not, so far as we are aware, present anything in common with a nucleus, except its capacity to divide, and in this connection such a capacity amounts to nothing, for the capacity to divide is one which is, of course, shared by every known centrosome. The extra-nuclear centrosome is generally in the form of a thick rod, often slightly curved, and sometimes presenting the appearance of being divided in the middle. The division of the blepharoplast does not appear, however, to take place through any transverse separation, which such appearances might suggest.

The nucleus in *T. lewisi* lies relatively very near the end of the body from which the flagellum projects. In consists of an outer less stainable area, and a large inner much more darkly staining globe, the *intra-nuclear centrosome* (karyosome nucleolus). The outer portion of the nucleus is often very distinctly bounded, and in such phases of the development as those represented in figs. 14-19 might certainly be said to possess a membrane. During the phase of the development we are now considering, the cells do not present any very definite granules in the cytoplasm, which is seen, both under examination during life with a dark ground illumination, and after proper fixation, to consist of a fine protoplasmic foam bounded on the outside by a denser and homogeneous layer.

Having thus briefly described the features of *T. lewisi* when in such a stage as that represented in fig. 1, it will be most convenient in proceeding to describe the passage of such forms through the phases of the cycle we have already outlined, and to consider the various divisional and other phenomena as they occur in relation to this cycle. The form of trypanosome represented in fig. 1 passes by simple growth into the large forms represented in figs. 11-18, and all the intermediate stages can be readily found stretching from the morphological condition represented in fig. 1 to that represented in fig. 17. Among such trypanosomes as those represented in figs. 8-17, two stages of metamorphosis are found to

occur. The first consists in the unequal budding of the intra-nuclear centrosome, so as to form what appears as a small nucleus, which becomes pushed off towards the free portion of the flagellum. This little mass, which consists of a small portion of the intra-nuclear centrosome and a small portion of the outer nuclear substance, appears subsequently to simply disappear. The process we have just described undoubtedly corresponds to the similar production of a degenerating nuclear bud in *T. equiperdum.* The second metamorphosis is constituted by the production of a body originating from the extra-nuclear centrosome. A portion of the substance of the extra-nuclear centrosome appears to pass round the adjacent vacuole, and to collect into a small mass on the side of the vacuole which faces the nucleus. This soon becomes completely detached, and passes away toward the nucleus through the cytoplasm. During its development the detached body becomes larger, and the outer portion of it stains less densely, but it is often possible to see a darkly staining bead at the centre of the growing mass (figs. 8-10). The body thus detached from the extra-nuclear centrosome may be found in all stages of transit from its original position to a close approximation to the nucleus (figs. 14-16). Having reached this latter position, it appears to remain for some time unchanged. The process here described in *T. lewisi* obviously corresponds to the similar detachment of a portion of the extra-nuclear centrosome, and its subsequent passage to the nucleus, which we have described in *T. equiperdum.* It also undoubtedly corresponds to the formation of the stainable band stretching between the extra-nuclear centrosome and the nucleus during the life cycle of *T. gambiense.* The nucleus itself usually at this period begins to show signs of division. Such division which is represented in figs. 18-19 takes place in a typical amitotic fashion; the intra-nuclear centrosome dividing like a drop as in *T. gambiense* and *T. equiperdum,* and the outer nuclear substance collecting round the two derivatives in the same manner.

As in *T. gambiense, T. equiperdum,* and *T. equinum,* so also in *T. lewisi* we have been absolutely unable to observe anything during the division of the nuclei, or during any other periods, which in the remotest degree suggests the presence of chromosomes. During division of *T. lewisi,* the intra-nuclear centrosome first elongates, then becomes dumb-bell shaped, and finally assumes the form of two large

globes widely separated from one another, and connected by a generally curved and tapering mass of substance which seems to have been simply drawn out between them. At the same time the outer nuclear substance collects about the diverging daughter elements, and finally separates along with them into two smaller masses, which, together with the new intra-nuclear centrosomes, eventually reproduce two complete and round nuclei, exactly like the parent nucleus only smaller. The process of nuclear division just described may be rapidly repeated; and at the same time the original trypanosome loses its characteristic form, and become rounded up so as to produce the well-known multi-nucleated masses such as those represented in figs. 12-25. In some cases, however, when the nucleus, in a specimen such as that represented in fig. 19 has divided, the trypanosomes may become longitudinally split as in fig. 39, and in these cases the nuclei may at the same time travel towards the extra-nuclear centrosome so as to occupy the position represented in fig 39. The ends of these division products may become detached from one another, and a very curious appearance result, represented in fig. 39. We think that the features of this form of division at the period we are discussing deserves particular attention, for the appearances produced when it occurs are indistinguishable from the figures given by Prowazek, and interpreted by him as conjugation.

We have, however, found nothing in relation to the nuclei, or any other structure in such cells, when in this condition, to suggest that the forms in question can be interpreted as conjugation. When such forms are produced, their future history appears to be this: Either the nuclei divide further, and the separation remains incomplete, the final product being one of the irregular multi-nucleated forms, or the fission is completed and the daughter cells, each after further nuclear divisions, produce fresh multi-nucleated masses.

We have referred to this process because of its obvious bearing upon the interpretation to be put upon the identical figures given by Prowazek. Our observations indicate that it is relatively a rare method of procedure, the more normal processes being the multiplication of the nuclei and the rounding up of the trypanosome to produce eventually the multi-nucleated masses. During this period, i.e., the time and after the body becomes detached from the extra-nuclear centrosome the history of this latter

structure in *T. lewisi* is difficult to follow. It certainly often enters into close contact with the nucleus before division as in figs. 15, 16, but it is frequently discernible after the nuclei have divided, as in fig. 20. In some of the resulting forms, moreover, when two, three, or four nuclei have been produced, the extra-nuclear-centrosomic derivative may sometimes still be observed lying between the nuclei, and apparently in close association with them. Whether the substance of the extra-nuclear-centrosomic derivative is directly absorbed by the nuclei, or merely disappears in the cytoplasm, the body in question sooner or later vanishes, and cannot be observed any further. The division of the nuclei of *T. lewisi* in the form we have just described is accompanied by the fission of the extra-nuclear centrosome, the fission of this latter body being generally accompanied by a movement towards the nucleus. It sometimes happens, however, that not only does the extra-nuclear centrosome move towards the nucleus, but the nucleus itself also moves towards the extra-nuclear centrosome. The advent of division of the extra-nuclear centrosome is marked by the development of the rod-like form into a flat disc, which perhaps through its thinness stains less darkly than the extra-nuclear centrosome when in a condition of rest (figs. 40-42.) The next phase is constituted by the collection of the staining material on opposite sides of the disc, and finally by the production in this way of two curved rod-like bodies on each side of the disc (figs. 41, 42). These new rod-like bodies constitute the new extra-nuclear centrosomes. They now rapidly diverge; it may be widely, showing at first a faint connection, which appears to be the remaining substance of the disc that has been simply drawn out. This connection rapidly disappears, the resulting extra-nuclear centrosomes having then the same appearance as those in the parent form; but they are naturally smaller. During the nuclear division at this period which result in the production of the multi-nucleated masses, the division of the extra-nuclear centroseme does not, so far as we have been able to see, result directly in a division of the flagellum or the body attached to its proximal end. During such phases in *T. lewisi*, the original flagellum and its bead remain unaffected, and apparently do nothing. When the extra-nuclear centrosome has divided, as in fig. 44, it is often seen that a small body is closely attached to it, appearing as if it had been separated from the extra-nuclear

centrosome. These little granules lie in the position from which new flagella finally arise, and it is consequently suggested that in *T. lewisi* the flagellum originates from a small fragment of the extra-nuclear centrosome, which becomes detached after the extra-nuclear centrosome has divided. This view of the method of procedure is further enforced by the fact that after the dissociation of the flagellum from the extra-nuclear centrosomes (which take place during the division of the latter bodies, see figs. 41-46), the original flagellum and its bead appears to be left, and is certainly finally shed in a degenerative condition, in the same way as the flagellum is cast off during the formation of the latent bodies in *T. gambiense* and *T. equiperdum.* From the vicinity of each of the new extra-nuclear centrosomes, and apparently from the granules budded off from these bodies, new and delicate flagella arise, and the multi-nucleated mass may assume in consequence appearances such as those represented in figs. 41-46.

It will be seen that the features of the phase we have now described, that is, the production of the large pointed forms, the passage of an extra-nuclear-centrosomic derivative to the nuclei, the subsequent division of the nuclei, the formation of new extra-nuclear centrosomes, the degeneration and disappearance of the old flagellum and the formation of new flagella in association with new extra-nuclear centrosomes, certainly correspond in a biological sense with the phases we have considered and described in relation to the production of the latent bodies in *T. gambiense* and *T. equiperdum.* It would seem, indeed, that the multiplication of the nuclei in the large multi-nucleated masses of *T. lewisi* correspond to the division in *T. equiperdum* after the passage of the extra-nuclear-centrosomic derivative to the nucleus. The subsequent history of the multi-nucleated forms is equally interesting in this comparative aspect. The nuclei and the extra-nuclear centrosomes may become multi-nucleated till there are 10, 15, or more of each in a single mass. The flagella become distributed on the periphery of such masses, and the mass finally separates by forming either a mulberry-like aggregate of round flagellated forms, or the fission proceeds in a slightly different manner, and a curious group of somewhat elongated forms may be produced, as fig. 29. In all these resulting forms, whether elongated or round, the morphological conditions are quite different from those of the

characteristic trypanosome form. The nuclei occupy a more or less central position. The body of the cell is short (fig. 28), or actually round (fig. 31), and the long delicate flagellum is quite free. When such forms are elongated, as in fig. 27, the flagellum and the extra-nuclear centrosomes lie together on one side of the nucleus, and the flagellum passes away from the vicinity of the extra-nuclear centrosome in an opposite direction to that of the nucleus. These forms arising from the ultimate breaking up of the multi-nucleated masses are thus seen to possess all the morphological characteristics of the latent bodies, which are produced after the corresponding cycle of internal changes in the case of *T. gambiense* and *T. equiperdum.* The derivatives of the multi-nucleated masses in *T. lewisi* thus correspond to the latent bodies of *T. gambiense* and *T. equiperdum.* In *T. lewisi,* however, the subsequent history of the latent bodies is far more easy to follow than in any case which we have hitherto been acquainted. *T. lewisi* in this respect constituting an admirable example for the further study of this important phase, which is less easy to follow in the development of the pathogenetic forms to which we have referred.

The changes which succeed in the small flagellated forms or latent bodies of *T. lewisi* are essentially similar to the transformation of the latent bodies of *T. gambiense* into the ordinary trypanosome form. The latent body elongates, the flagellum at first passing directly away from the surface of the body and in a direction opposite to that in which the nucleus lies in respect to the extra-nuclear centrosome (fig. 31). After a time the extra-nuclear centrosome migrates to one end of the cell body (fig. 32), and the flagellum is apparently drawn over the surface of the body after it. This portion of the flagellum which remains attached to the cell forms, as it were, the 'Anlagen' of the future undulating membrane. The further development is simple, the body elongating and enlarging into the ordinary trypanosome shape, as in figs. 33-38. When the form of small trypanosome, such as that represented in fig. 38, has been assumed, the cells again enter into division, as may be seen in figs. 34-37, and through the process of growth and further fissions gradually pass back again to the forms with which this description started (fig. 1).

In briefly considering the foregoing observations upon the life history of *T. lewisi,* the most striking biological feature which emerges is the obvious similarity that exists between the successive phases

presented by *T. lewisi*, and the homologous phases occurring in the life cycles of *T. gambiense* and *T. equiperdum*. In each of these three cases the 'trypanosome form' multiplies through fission until an interaction takes place between the extra-nuclear centrosome and the nucleus. This interaction may be succeeded again by simple fission, as in *T. equiperdum*. Possibly this is also the case in *T. gambiense*, while in *T. lewisi* it is followed by a series of rapid nuclear divisions resulting in the formation of the characteristic multi-nucleated masses. These differences, however, appear to be mere specific differences, of quite minor importance, which simply help to characterise in a specific sense the particular parasites we have considered. The divisions following the interaction between the extra-nuclear centrosome and the nucleus are, however, succeeded by a complete change of form, and by the assumption of the peculiar morphology of the round flagellated 'latent body.' The fact that in *T. equiperdum* the latent bodies possess two flagella has probably a profound morphological significance, but it seems to be inappropriate at the present time, and in view of future work, to enter into a consideration of its actual significance. The details of the structure of the latent bodies, and their passage into the trypanosome form, is a matter which, although apparently simple in *T. lewisi*, is one which must receive further study. It is, for example, not at present clear in what way the two flagella of the latent body of *T. equiperdum* behave during this process. It becomes clear, when we consider the observations on the three forms to which we have referred, that during the life cycle in the blood the different phases in this cycle may become prolonged or shortened relatively with respect to one another.

Thus the stage in *T. gambiense*, where one or two fissions possibly follow, the interaction between the extra-nuclear centrosome and the nucleus is prolonged in the case of *T. equiperdum* into a period where certainly several divisions take place, and this same period is again prolonged and rendered specifically characteristic in the case of *T. lewisi* by the production of the large multi-nucleated masses.

In this connection it seems also to be a very striking fact that, whereas in the pathogenetic forms *T. gambiense* and *T. equiperdum* the phases of the life cycle as they appear among the trypanosomes do so nearly simultaneously among all the parasites existing in the blood at a particular time, and thus mark successively

the stages of the infection, in the non-pathogenetic form *T. lewisi* all the stages of the life cycle may be present and represented by different parasites which are found in the blood at the same time. This difference is perhaps what might have been expected. Such forms as *T. lewisi* are usually present in the animals they inhabit in large numbers for weeks, or even months; whereas among the pathogenetic varieties the parasites are numerous in the blood for only relatively short periods, the phases of the life cycle being here apparently adapted to the varying conditions of the host.

In this way we find that the parasites in such forms either multiply without limit, and by their action rapidly kill the host, or they periodically disappear from the altered blood in the form of latent bodies, and only reappear, it may be, after a very considerable time.

We have referred, in dealing with *T. gambiense* and *T. equiperdum*, to the fact that we have been quite unable to make anything of the arbitrary distinction which has come into vogue since the publications of Schaudinn between the so-called males, females, and any different forms. These seem to us to be either mere varieties of size, or, where morphological distinction is obtained, examples that have been taken from different parts of the life cycle.

The same results in relation to this matter have been enforced by the study of *T. lewisi*. Moreover, the terms male and female have, biologically speaking, always a strict and obvious reference to two varieties of cells which conjugate, or gametes, and to use terms of this type in reference to mere varieties of size, or to the morphological characters of different phases in a life cycle where no ordinary conjugation has hitherto been found, seems to us to be in the highest degree misleading and erroneous.

PLATE II

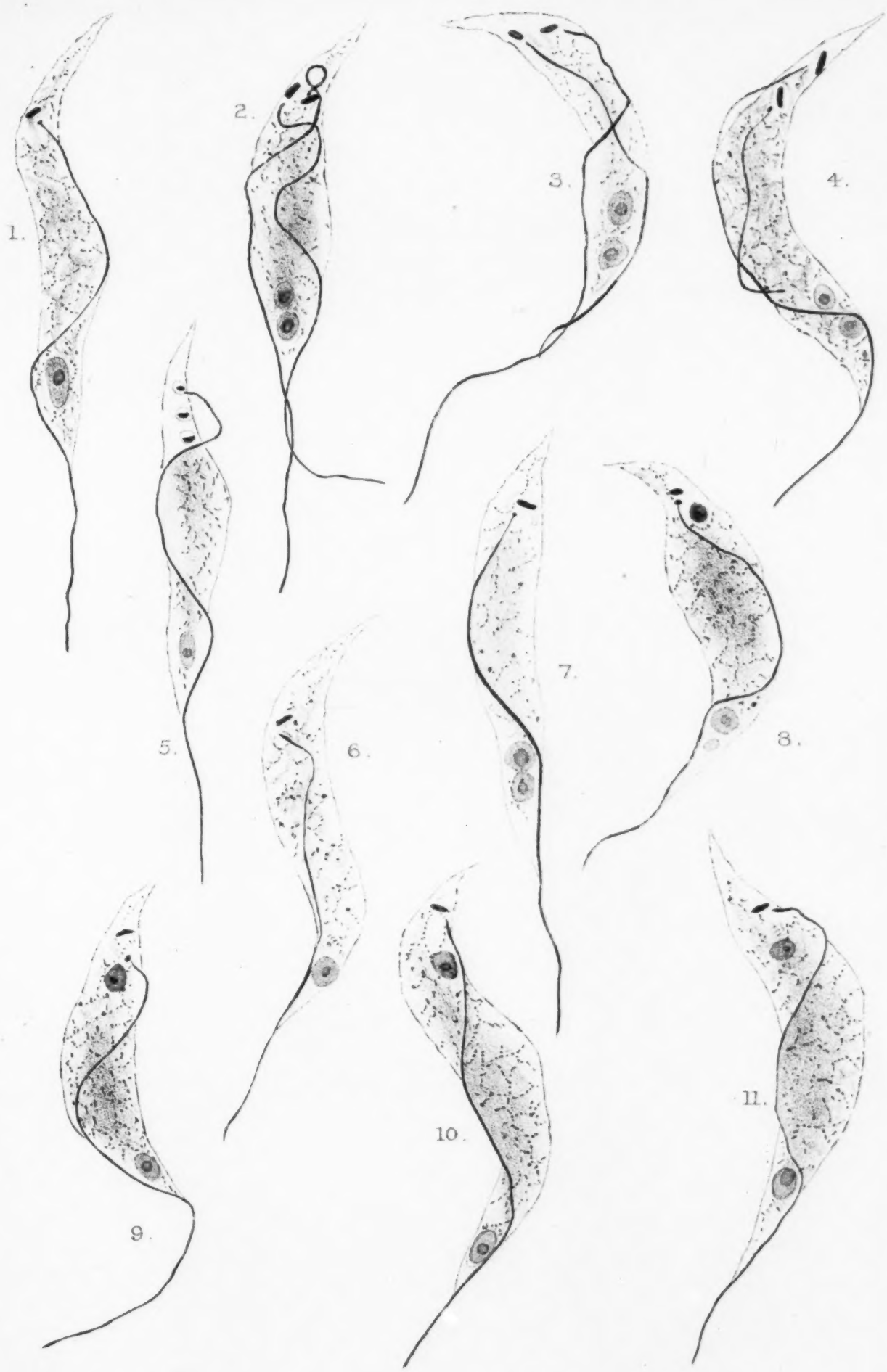
T. lewisi

Figs. 1-6.—Stages of rest and division of the nucleus, the extra-nuclear centrosome, and the development of new flagella.

Fig. 7.—Stage in the division of the nucleus.

Fig. 8.—Formation of a small nuclear body which is thrown off from the nucleus and production of a large mass from the extra-nuclear centrosome.

Figs. 9, 10, 11.—Further stages in the development of the body derived from the extra-nuclear centrosome, and its passage towards the nucleus.



J.E.S.M.del. Huth, Lith^r London.

PLATE III

T. lewisi

Figs. 12, 13, 14, 15, 16, 17.—Stages in the passage of the body developed from the extra-nuclear centrosome towards the nucleus. Figs 12 and 17 show the degeneration of the small mass detached from the nucleus.

Figs. 18, 19.—Stages of the division of the nucleus.

Fig. 20.—Partly rounded form with two extra-nuclear centrosomes, and the body derived from the extra-nuclear centrosome.

Fig. 21.—Rounded mass produced after the division of the nucleus, and extra-nuclear centrosome.

Fig. 22—Division of a nucleus in a large rounded form.

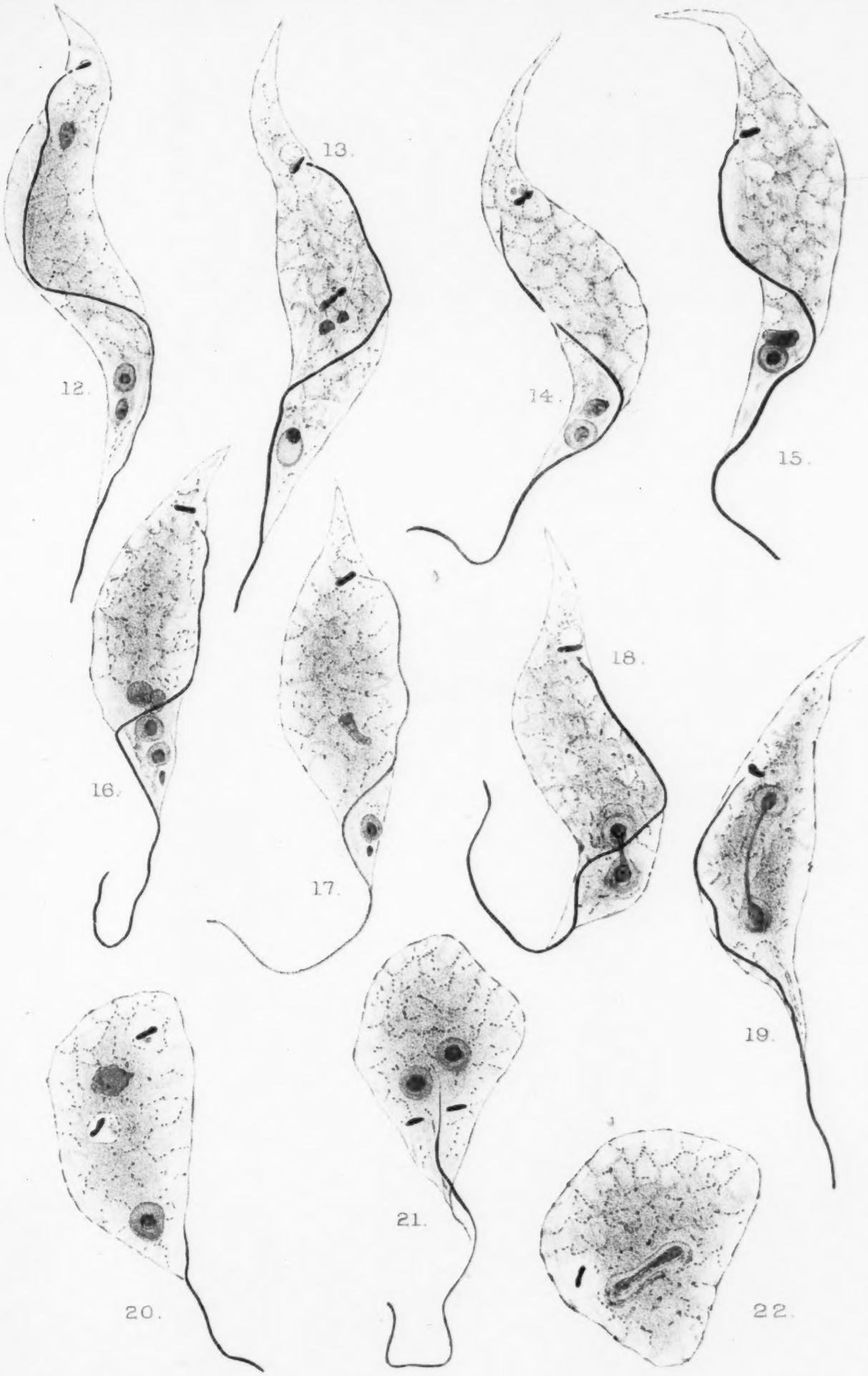


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PLATE IV

T. lewisi

Fig. 23.—Large form showing two nuclei and two extra-nuclear centrosomes.

Figs. 24, 25.—Large multi-nucleated masses, fig. 24 showing division of one of the nuclei.

Figs. 26, 27, 28, 29, 30.—Breaking up of the large multi-nucleated masses into forms equal to the latent bodies of *T. gambiense* and *T. equiperdum.*

Figs. 31, 32, 33, 34, 35, 36, 37, 38.—Stages of transformation of the latent bodies into ordinary trypanosomes.

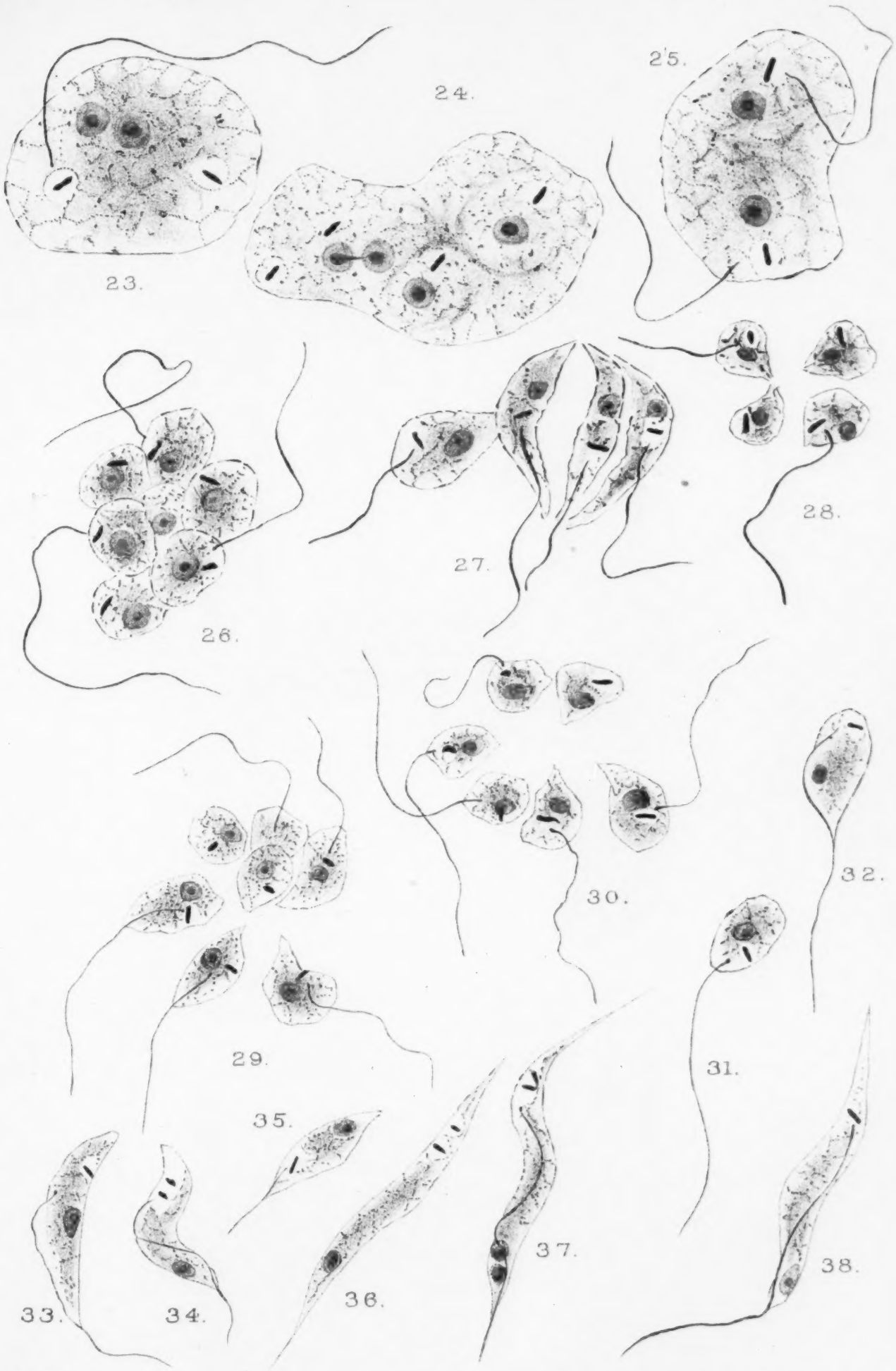

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PLATE V

T. lewisi

Fig. 39.—Division which at first sight suggests an act of conjugation.

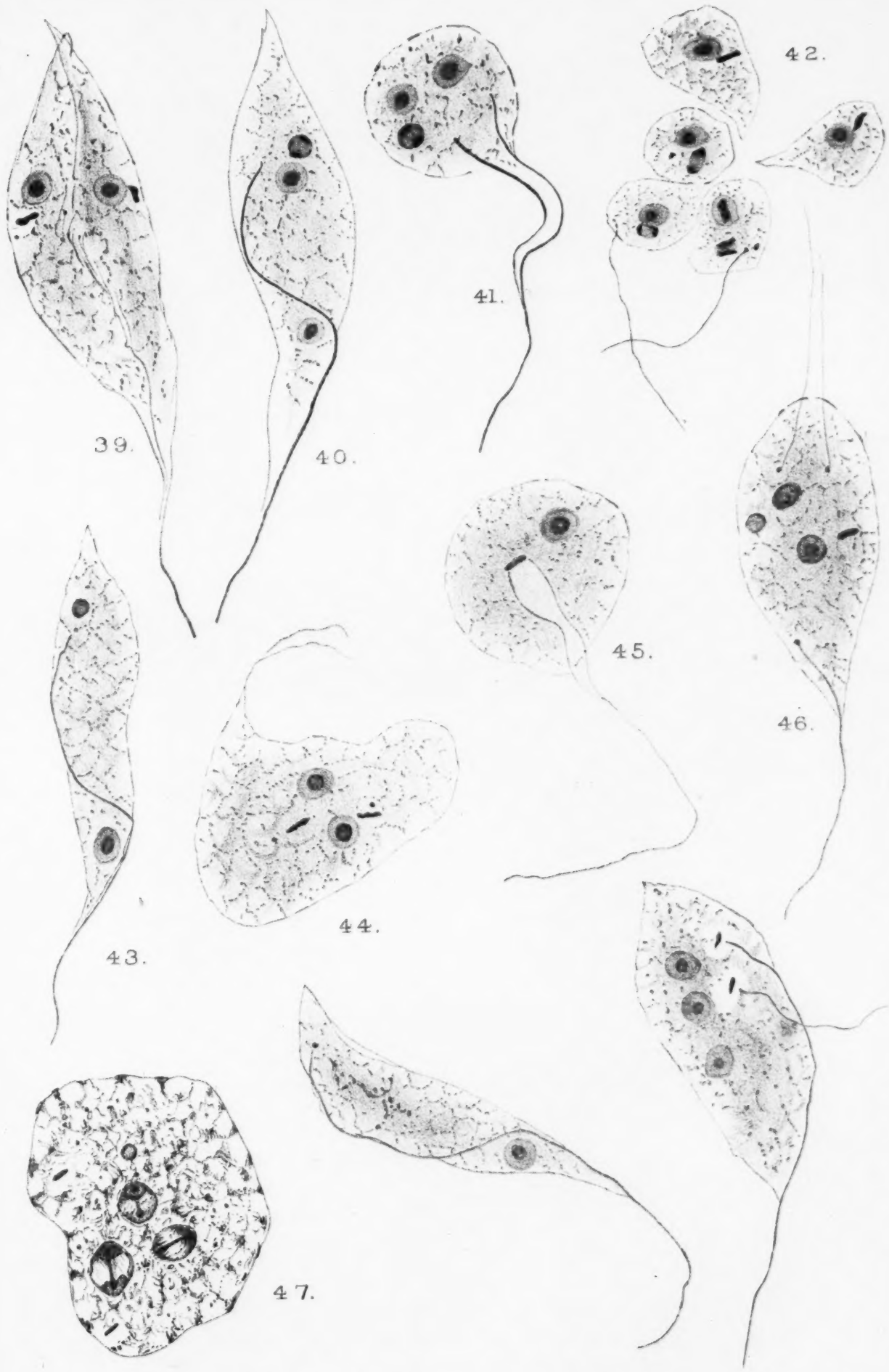
Figs. 40, 41, 42, 43.—Forms showing details of the division of the extra-nuclear centrosome.

Fig. 44.—Form showing detachment of small bodies from the extra-nuclear centrosome.

Figs. 45, 46.—Details of the origin of the flagella.

Fig. 47.—Division of the nuclei in a multi-nucleated mass.

Figs. 48, 49.—Unusual division of the nuclei.



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NOTES ON THE EFFECTS OF THERAPEUTIC AGENTS ON TRYPANOSOMES IN RESPECT TO (*a*) ACQUIRED RESISTANCE OF THE PARASITES TO THE DRUG, AND (*b*) CHANGES IN VIRULENCE OF THE STRAINS AFTER ESCAPE FROM THE DRUG

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A. DEVELOPMENT OF DRUG-PROOF STRAINS

It is a well-established fact in the therapeutics of trypanosomiasis that, with each fresh recurrence of the parasites in any case, these become more resistant to the drug employed, and more difficult to drive out of the peripheral circulation.

This is not only the fact in any given case, but also as was first shown by Ehrlich* in the case of rats treated by atoxyl; the parasite itself, apart from the individual host, becomes resistant to the drug, and, when passed to fresh animals of the same species, reaches finally a condition in which it is resistant or 'fast' to the particular drug. This condition Ehrlich expressed by the term 'Atoxyl-fest' in the case of trypanosomes which had become refractory to the action of atoxyl.

* Berl. Klin. Wochensch., 1907, p. 33.

We have observed similar effects, and they have also been noted by Plimmer and Thomson* in the case of atoxyl. Ehrlich† also found a developed resistance in the case of certain of the anilin colours used by him as trypanocides, the colours experimented with being parafuchsin, trypan-red and trypan-blue. The special point which we believe is shown in regard to this resistance by the experiments here recorded is that it only holds for the species of animal in which it has been established, and disappears when the fast strain of trypanosomes is passed into another species of animal.

T. brucei was used in the experiments, and two distinct strains were used: (*a*) a strain which was atoxyl-fast in mice, for which we are indebted to the kindness of Prof. Ehrlich, and (*b*) an atoxyl-proof strain in the rat, obtained by ourselves after eight relapses. The results with the first of these strains are given in Table I, those with the second strain in Table II.

TABLE I

Strain atoxyl-fast in mouse as shown by first three experiments, but not fast in rat and dog as shown by subsequent experiments.

Experiment	Treatment	Duration of life	Examinations
1. Mouse (84)	0·5 c.c. of 2 per cent. acetylated atoxyl‡ repeated three times	8 days	Trypanosomes seen all the time, apparently diminished after second injection
2. Mouse (85)	As above	7·5 days	As above
3. Mouse (92)	,,	6 days	,,
4. Rat (265)	0·5 c.c. of 5 per cent. solution of atoxyl	19 days	Trypanosomes disappeared and reappeared in twenty days
5. Rat (266)	As above	24 days	,,
6. Dog (312)	10 c.c. of 10 per cent. solution of acetylated atoxyl in two injections	29 days	Trypanosomes disappeared and reappeared on twenty-sixth day

* Proc. Roy. Soc., Vol. 79B, 1907, p. 504.

† *Loc. cit.*

‡ A supply of this substance was kindly sent us by Professor Ehrlich.

TABLE II

Strain atoxyl-fast in the rat as shown by first three experiments, but amenable to atoxyl in the mouse and dog as shown by the other experiments.

Experiment	Treatment	Duration of life	Examinations
1. Rat (289)	1 c.c. of 5 per cent. atoxyl	9 days	Trypanosomes were found on all but one day
2. Rat (292)	As above	11 days	Trypanosomes present all the time
3. Rat (298)	1 c.c. of 5 per cent. atoxyl, twice repeated	10 days	Trypanosomes present all the time
4. Mouse (94)	0·5 c.c. of 2 per cent. acetylated atoxyl given three times	23 days	Trypanosomes disappeared in twenty days
5. Mouse (97)	As above	26 days	Trypanosomes disappeared in twenty-three days
6. Dog (316)	10 c.c. of 10 per cent. acetylated atoxyl	19 days	Trypanosomes disappeared and reappeared in fourteen days

B. VARIATIONS IN VIRULENCE IN FRESH ANIMALS OF STRAINS WHICH HAVE REAPPEARED AFTER TREATMENT WITH DIFFERENT DRUGS

In our work on the treatment of Ngana (*T. brucei*) by atoxyl and mercury salts in rats, we have observed that when the parasites had reappeared after atoxyl treatment, and were then passed on into a fresh animal, they had acquired increased virulence in the process and caused death more rapidly than when passed on from animal to animal without any treatment. Accordingly a series of experiments

with animals infected with *T. brucei*, *T. gambiense* and *T. dimorphon* was instituted specially to make observations on this point. In the case of the infections with *T. brucei* it was found that the virulence increased after attack with atoxyl, but was diminished after trypan-red. A slight increase in virulence was seen in the few experiments done with *T. dimorphon*, and a slight decrease in the case of *T. gambiense*, but the number of experiments and alteration in the virulence are both too small to warrant a definite conclusion in the case of these two species. The number of experiments with *T. brucei* is larger, and we are of opinion that this parasite after escape from atoxyl is, in the majority at least of the cases, more virulent than before treatment.

Previous experiments, without treatment with any drug, gave as the average duration of life after appearance of infection in the case of each strain as follows:—

T. brucei	6·3 days	Average of	146 rats
T. dimorphon	17·6 ,,	,,	56 ,,
T. gambiense	28·7 ,,	,,	36 ,,

No sudden alteration in virulence was found in the case of any of the strains. This had been specially watched for in the case of the strain of *T. brucei*, which had been under observation from 4th November, 1906, till 16th July, 1907; as stated, no increase of virulence was seen in the strain passed in the usual way from animal to animal without coming under the influence of any drug.

In order to infect the animals, an injection subcutaneously of 0·4 c.c. of infected blood, containing 6 to 8 parasites to the field (Zeiss, ocular 4, objective DD), was given in each case.

The results are shown in the following tables, which show the day of reappearance of trypanosomes in the peripheral circulation after their disappearance under the influence of the drug mentioned in each case, the number of animals then infected by the recurrent strain from the drug, the duration of life under this strain, and the increase in virulence marked +, or decrease marked — .

TABLE III

Virulence of *T. brucei* recurrent after atoxyl treatment.

Day of relapse	No. of animals infected with recurrent strain	Average duration of life in days	Change of virulence
	(Untreated rats 146)	(6·3)	
23rd day	4 rats	4·6	+ 1·7
29th ,,	7 ,,	5·0	+ 1·3
19th ,,	2 ,,	6·0	+ 0·3
24th ,,	4 ,,	3·8	+ 2·5
26th ,,	3 ,,	6·8	− 0·5
38th ,,	7 ,,	4·2	+ 2·1
25th ,,	2 ,,	5·0	+ 1·3
19th ,,	3 ,,	6·0	+ 0·3
29th ,,	5 ,,	7·5	− 1·2
21s ,,	8 ,,	4·5	+ 1·8
35th ,,	6 ,,	4·0	+ 2·3
31st ,,	3 ,,	6·8	− 0·5

Taking the averages of the whole numbers of untreated and treated rats, we have :—

Untreated rats	146	Average duration of life	6·3 days
Post-atoxyl-treatment rats	54	,, ,, ,,	5·1 ,,
		Mean increase of virulence	1·2 ,,

The same strain of *T. brucei* treated with trypan-red in three experiments in which 11 rats were infected from recurrences after this drug, showed on the other hand a considerable decrease in virulence of the strain, as shown in the following table.

TABLE IV

Virulence of *T. brucei* recurrent after trypan-red treatment.

Day of relapse	No. of animals infected with recurrent strain	Average duration of life in days	Change of virulence
	(146 rats untreated)	(6·3)	
7th day	4 rats	15	− 9·7
11th ,,	3 ,,	9	− 2·7
5th ,,	4 ,,	11	− 4·7

Untreated rats	146	Average duration of life	6·3 days
Post-trypan-red-treatment rats	11	,, ,, ,,	11·9 ,,
		Mean decrease of virulence	5·6 ,,

The strain of *T. dimorphon*, after treatment with atoxyl, gave the following results in five experiments with 18 rats:—

TABLE V.

Virulence of *T. dimorphon* recurrent after atoxyl treatment.

Day of relapse	No. of animals infected with recurrent strain	Average duration of life in days	Change of virulence
	(Untreated rats 56)	(17·6)	
29th day	5 rats	14·5	+ 3·1
42nd ,,	2 ,,	19·0	− 1·4
34th ,,	4 ,,	11·0	+ 6·6
28th ,,	3 ,,	19·0	− 1·4
26th ,,	4 ,,	16·0	+ 1·6

Untreated rats	56	Average duration of life	17·6 days
Post-atoxyl-treatment rats	18	,, ,, ,,	15·3 ,,
		Mean increase in virulence	2·3 ,,

In four experiments with the strain of *T gambiense*, in which 20 rats were used, the following decrease in virulence was observed, but as stated above a much larger number of experiments would be required in order to definitely decide the point, for this much slower species of trypanosome:—

TABLE VI

Virulence of *T. gambiense* recurrent after atoxyl treatment.

Day of relapse	No. of animals infected with recurrent strain	Average duration of life in days	Change of virulence
	(36 rats untreated)	(28·7)	
59th day	6 rats	32	− 3·3
62nd ,,	7 ,,	30	− 1·3
54th ,,	4 ,,	39	− 10·3
68th ,,	3 ,,	31	2·3

Untreated rats	36	Average duration of life	28·7 days
Post-atoxyl-treatment rats	20	,, ,, ,,	32·5 ,,
		Mean decrease in virulence	3·8 ,,

It may, perhaps, for clearness be emphasized that none of the animals mentioned in the Tables (III to VIII) received any treatment whatever. The comparisons are entirely of the virulence of the strains in what might be described as their natural condition, and when they have reappeared after a drug and are passed into a fresh host of the same animal where they are not further treated.

OBSERVATIONS ON THE ACIDITY AND ALKALINITY OF THE BLOOD IN TRYPANOSOME INFECTIONS

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Yakimoff[1] in his publications dealing with the changes of blood during trypanosome infection states that the alkalinity of blood decreases as the disease progresses. He used for his estimations von Limbeck's[2] method, whereby the serum alkalinity is measured against litmus. This method, however, does not indicate the true alkalinity of the serum, as the results are affected by the increase of the acidity and the carbon dioxide present in the blood.

A method which practically eliminates these errors has been described recently by Moore and Wilson,[3] who estimate the alkalinity of the ash after incineration of the blood. We were able to show, with their method, that during infections with *T. brucei* and *T. equiperdum* the acidity of the blood serum increases, whereas the alkalinity of the blood apparently remains constant.

B. Moore's and F. Wilson's technique was adopted, phenolphthaleine being used as an indicator for alkalinity, and dimethyl-amido-azo-benzol (referred to as dimethyl for brevity) for acidity. In addition titrations were made with Congo red, as this does not indicate organic acids such as amido acids.[4]

It is remarkable that in cases where both indicators were used, the acidity against Congo red was lower than against phenolphthaleine. This difference becomes more marked as the infection progresses, a fact which seems to suggest that trypanosome infection causes an increase of amido acids in the blood.

To eliminate the error which might be produced by the CO_2 in the breath, to which Moore and Wilson allude in their paper, special

precautions were taken. The mouth-part of the pipette contained KOH between two layers of cotton wool, so that all the CO_2 of the breath was absorbed by the potassium hydroxide.

The blood was collected in small test tubes and left standing over night in an ice chest; then the serum was separated from the clot and used for the estimation of acidity. For the estimation of the alkalinity, the blood was collected in a platinum crucible and incinerated. All the glass vessels used in this work were immersed in strong hydrochloric acid for three days, and then in distilled water for the same length of time, so as to avoid the error which might be produced by the alkalinity of the glass.

EXPERIMENT No. 303.—Rabbit, male, inoculated on 10th February, 1908, with *T. equiperdum*. On 12th February the acidity was 0·03015* (phenolphthaleine); on 24th February the acidity had increased to 0·03255 (phenolphthaleine), 0·03195 (congo red), the alkalinity was 0·02895. The animal was frequently examined and kept under observation until 7th April, when the acidity had reached 0·03495 (phenolphthaleine), 0·03345 (congo red), and the alkalinity 0·03015.

EXPERIMENT No. 305.—Rabbit, female, inoculated on 10th February, 1908, with *T. equiperdum*. On 24th February the acidity was 0·03285 (phenolphthaleine), 0·03225 (congo red), and the alkalinity 0·02865. On 7th April the acidity had reached 0·02865 (phenolphthaleine), 0·03405 (congo red), and the alkalinity 0·02925. It is interesting to note that on 31st March the acidities against congo red and phenolphthaleine were the same, 0·03465.

EXPERIMENT No. 307.—Guinea-pig, female, inoculated on 24th February, 1908, with *T. brucei*. The first alkalinity and acidity estimations were made on 26th February, when the acidities were found to be 0·03105 (phenolphthaleine), 0·03075 (congo red), and the alkalinity 0·03045. On 11th March the animal was found to be swarming with parasites, and the acidity estimation on 14th March gave 0·03435 (phenolphthaleine), 0·0327 (congo red), and the alkalinity 0·03000. The animal died on 18th March of typical trypanosomiasis. The acidity estimation made twenty minutes after death gave 0·03615 (phenolphthaleine), and 0·03480 (congo red).

EXPERIMENT No. 309.—Guinea-pig, female, inoculated on 24th February, 1908, with *T. brucei*. The first acidity estimation was made on 26th February, and found to be 0·03195 (phenolphthaleine), 0·03175 (congo red), and the alkalinity 0·03030. The animal showed trypanosomes on the same day. On 7th April the acidity had reached 0·03465 (phenolphthaleine), 0·03450 (congo red), and the alkalinity 0·02925; the animal was then heavily infected.

EXPERIMENT No. 310.—Guinea-pig, female, inoculated on 24th February, 1908, with *T. brucei*. On 26th February the acidity was 0·03225 (phenolphthaleine), 0·03210 (congo red), and the alkalinity 0·02895. On 8th March the acidity was 0·03405 (phenolphthaleine), 0·03300 (congo red). A fall in the acidity was noticed on 14th March. The acidity reached 0·03195 (phenolphthaleine), and the same also for congo red. Afterwards the acidity started slowly to increase and became on March 23rd 0·03510 (phenolphthaleine), 0·03375 (congo red), and the alkalinity 0·02985. On 7th April the acidity had reached 0·03585 (phenolphthaleine), 0·03495 (congo red), and the alkalinity 0·03045.

* Expressed in fractions of Normal.

EXPERIMENT No. 311.—Guinea-pig, male, inoculated on 24th February, 1908, with *T. brucei*. On 24th February the acidity was found to be 0·03245 (phenolphthaleine), 0·03225 (congo red), and the alkalinity 0·03000. The last estimation was made on 17th March, and the animal died on 19th March. The animal was then slightly infected, and the acidity found to be 0·03300 (phenolphthaleine), 0·03255 (congo red), and the alkalinity 0·03000. The cause of the death of this animal was pneumonia.

EXPERIMENT No. 314.—Rabbit, female, inoculated on 14th March, 1908, with *T. brucei*. On the day of inoculation the acidity was found to be 0·03075 (phenolphthaleine), 0·03030 (congo red), and the alkalinity 0·02850. On 23rd March the acidity was found to be 0·03300 (phenolphthaleine), 0·03195 (congo red), and the alkalinity 0·03060. On the same day, three young ones were born which soon died. The acidity on 26th March had dropped down to 0·03240 (phenolphthaleine), 0·03225 (congo red), and the alkalinity 0·02850. From this day the acidity increased slowly, and on 7th April was 0·03345 (phenolphthaleine), 0·03285 (congo red), and the alkalinity 0·03015. The animal died on 9th February. This animal was positive all the time, starting from 17th March.

EXPERIMENT No. 351.—Rabbit, female, inoculated on 14th March, 1908, with *T. brucei*. The acidity was then 0·03120 (phenolphthaleine), 0·03075 (congo red), and the alkalinity 0·02940. On April 7th the acidity had reached 0·03375 (phenolphthaleine), 0·03315 (congo red), and the alkalinity 0·02910.

EXPERIMENT No. 316.—Rabbit, male, inoculated on 14th March, 1908, with *T. brucei*, showing an acidity of 0·03180 (phenolphthaleine), and 0·03075 (congo red), and an alkalinity of 0·02880. When examined on 31st March, the acidity had reached 0·03375 (phenolphthaleine) 0·03210 (congo red), and the alkalinity 0·03000.

EXPERIMENT No. 317.—Guinea-pig, female, inoculated on 14th March, 1908, with *T. brucei*. The acidity was then 0·03150 (phenolphthaleine), 0·03105 (congo red), and the alkalinity 0·02895. The animal died on 8th April of typical trypanosomiasis. On 7th April the acidity had reached 0·03585 (phenolphthaleine), and 0·03360 (congo red), when the alkalinity was 0·02850.

I should like especially to draw attention to this experiment, in which both the increase of the total acidity and also that of the amido acids is very marked.

The following table gives in full the changes in acidity and alkalinity during an experimental infection:—

EXPERIMENT No. 304.—Rabbit, male, inoculated 10th February, 1908, with *T. equiperdum*.

Date	Acidity to phenolphthaleine	Acidity to congo red	Alkalinity to dimethyl
12/2/08	0·03135	—	—
14/2/08	0·03195	—	—
18/2/08	0·03225	—	0·02895
20/2/08	0·03285	0·03195	0·02880
24/2/08	0·03240	0·03210	0·02880
27/2/08	0·03240	0·03225	—
2/3/08	0·03255	0·03210	0·02895
4/3/08	0·03300	0·03210	0·02865
8/3/08	0·03255	0·03225	—
10/3/08	0·03285	0·03210	0·02895
14/3/08	0·03255	0·03210	0·02865
17/3/08	0·03255	0·03255	0·02880
19/3/08	0·03285	0·03225	0·02910
23/3/08	0·03315	0·03225	0·02985

N.B.—Trypanosomes examinations were made daily. The animal died on 25/3/08, when the *post-mortem* showed a more or less marked trypanosomiasis.

The following table shows the acidity and alkalinity of normal rabbit serum :—

Sex	Acidity to phenolphthaleine	Acidity to congo red	Alkalinity to dimethyl
F.	0·03175	—	—
M.	0·03225	—	—
F.	0·03075	—	—
F.	0·03180	—	—
F.	0·03075	—	—
F.	0·03120	—	—
M.	0·03090	—	—
F.	0·03120	—	—
F.	0·03015	—	—
F.	0·03175	—	—
F.	0·03075	—	—
F.	0·03120	—	—
M.	0·03015	—	—
F.	0·03150	—	—
F.	0·03075	0·03075	0·02895
M.	0·03175	0·03105	0·02850
F.	0·03075	0·03120	0·02895
F.	0·03195	0·03060	0·02955
F.	0·03175	0·03030	0·02865
F.	0·03050	0·03050	0·03045
F.	0·03120	0·03050	0·03000
M.	0·03030	0·03015	0·02880
F.	0·03075	0·03030	0·02850
M.	0·03180	0·03075	0·02880
F.	0·03120	0·03075	0·02940
	Average 0·03115	Average 0·03064	Average 0·03025

CONCLUSIONS.

I. It is evident, that in experimental trypanosomiasis infection (*T. brucei* and *T. equiperdum*), the acidity of the blood increases.

II. The increase of the acidity is probably due to the production of amido acids through, or by the trypanosomes, i.e., the acids might be either secreted by the parasites or produced by the action of the parasites on the proteins of the blood serum. In the latter case, the amido acids would be broken up through hydrolysis from the proteins into simpler polipeptids.

III. It is possible that the increase of acidity might be of assistance in the diagnosis of a typical case of trypanosomiasis, where the parasites have disappeared for some length of time from the blood circulation.

IV. These experiments suggest that in trypanosome treatment effort should be made to neutralise the increased acidity of the blood, as this might prove of additional assistance in making the blood a less favourable medium for their development.

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CONTRIBUTIONS TO THE MORPHOLOGY AND LIFE HISTORY OF *PIROPLASMA CANIS*

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Piroplasma canis was discovered by Piana and Galli-Valerio in the year 1895,[20] and, on account of its wide distribution and the ease with which experimental infection can be transmitted from dog to dog, this parasite has been the subject of extensive study. It seems unnecessary to give a complete review of the literature, especially since Nuttall and Graham-Smith,[17-19] and more recently Christophers,[3] have given fairly complete bibliographies.

Additional interest has been attached to Piroplasmata in general since the appearance of Schaudinn's[21] work, in which he mentions (p. 428) Kossel's and Weber's observation, and suggests that *Piroplasma* may pass through a life cycle similar to that of *Halteridium*. However, very little evidence in support of this hypothesis has been brought forward by later workers.

The parasite, on which the following observations were based, was obtained from Professor Uhlenhuth, of Berlin, to whom we have pleasure in expressing our indebtedness. The strain has been kept going in pups and dogs by means of simple inoculation. In our hands it has shown itself very virulent, even in the case of full-grown dogs, and no animal survived the infection. Young dogs showed parasites, in scanty number, two to three days after an intra-peritoneal injection of 1 to 2 c.c. of heavily-infected blood; their number increased very slowly during the following 24 hours; after this a rapid increase in the number of parasites set in. Within 40 hours after the first

appearance of the parasites, the peripheral and especially the blood of the organs was usually teeming with them, and the animal succumbed to the infection. The course of the disease in full-grown dogs was somewhat modified, as the parasites only appeared after a prolonged incubation period, and never in such large numbers as in young animals.

The clinical feature of this disease has been dealt with in full by previous workers. It is a noteworthy fact that in nearly all our cases haemoglobinuria was more or less pronounced, the urine being frequently of a dark port wine colour. Only in very few cases was jaundice well marked.

Technique. All our observations were made on wet films using Breinl's methods. The blood-smears were fixed in strong Flemming's solution, and afterwards stained with safranine and methylene blue, according to Breinl's method, or his modification of Heidenhain,[15] using as counter-stain a dilute solution of Bordeaux red. By this means the cytological details of the parasites were well preserved, an attainment which is impossible by any dry film method.

EARLY FORMS OF PARASITES IN THE BLOOD

The early forms in the blood are usually very large and irregular, frequently exhibiting pseudopodia of varying form and size (figs. 1-5). Some of these processes are so fine that they simulate flagella, and at times small particles of protoplasm appear to become detached; but in most instances a very fine band may still be detected connecting these little masses with the parasite. The protoplasm consists of a fairly coarse spongioplasm ('Schaumplasma'), containing fine, bluish staining granules, embedded in its substance. At this stage the parasites usually possess a single nucleus, in the form of a small, dense, darkly staining mass of chromatin, which is sometimes surrounded by a vacuole, filled with lightly staining substance. The division of these forms is by simple fission. The nucleus of the mother cell elongates, and afterwards separates into two halves which move further apart; meanwhile the parasite itself increases in size, and eventually divides into two daughter cells (figs. 2-4). This process goes on very rapidly.

In the early forms very rarely a second smaller chromatic mass is

present (fig. 5), which may be connected with the main nucleus by a fine darkly staining line. If Breinl's stain is used this smaller nucleus usually takes a dark purplish-blue colour, whereas the nucleus stains dark red. This difference is noticeable, however, only in well stained specimens.

The division of the later forms proceeds in a different way. The small nucleus together with the chromatic line usually divides first. A median cleft afterwards appears, which extends between the two small nuclei. In the meanwhile the large nucleus elongates, and finally separates into two equal halves. The two daughter cells then become separate (figs. 6-9). This division results in the formation of two pear-shaped parasites.

At this stage of the infection, round parasites now and again reproduce by budding, a process which becomes more frequent as the infection advances. The nucleus throws out a portion of its chromatin, which moves outwards, but remains connected with it by means of a thick band. As this chromatic mass approaches the periphery of the parasite, the cytoplasm bulges out from the surface and concentrates itself around the terminal enlargement of the chromatic band. The latter structure becomes thinner and finally breaks. The connection of the bud with the main mass becomes in the meanwhile less and less extensive, and finally the bud is detached (figs. 10-12). Very often two buds are formed at the same time in a similar manner (figs. 13-16). A large number of buds as described by Kinoshita[7] have never been distinctly observed by us.

Schaudinn[21] and Lühe[13] were the first to draw attention to the presence of a small nucleus in *Piroplasma*. This discovery has been confirmed by different workers. Schaudinn named the second nucleus a blepharoplast, and most of the later workers adhere to this view, without, however, producing any evidence in support of it.

Our observations show that but few binucleate parasites are present at an early stage of the infection. This small nucleus then arises from the large one, usually at a later stage of the disease. Different phases of this process may sometimes be seen in one red corpuscle containing several parasites (figs. 17, 18, 42). The nucleus, which at this stage is surrounded by a vacuole, buds off a small part of its substance, which moves to the edge of the vacuole, often leaving a thin connecting line behind.

LATER FORMS OF PARASITES IN THE BLOOD

As the infection advances, the parasites undergo marked changes, and only now and again large amoeboid forms are seen. The parasites diminish in size, and are frequently pear-shaped. The protoplasm, which at first is a typical 'Schaumplasma,' becomes much denser in structure. The percentage of binucleate forms increases, and many free forms are encountered.

A peculiar feature of this stage is the detachment of small parts of the cytoplasm in a definite way. At one side of the cell appears a vacuole, which increases in size and enlarges within the parasite, until the protoplasm is almost separated into two unequal parts, which finally become separate. The smaller part is entirely cytoplasmic in nature (figs. 22-26).

Owing to the rapidity with which multiplication takes place, the nuclear details become very irregular, and frequently a second division commences before the completion of the first (figs. 20, 51-52).

The nucleus of the round forms is usually surrounded by a vacuole (fig. 33). The division is by simple fission, in which the nucleus divides with the vacuole (figs. 33-38).

Sometimes the parasites assume a signet-ring form, a large vacuole occupying the middle of the cell, the nucleus which lies at the periphery often dividing (fig. 21).

The usual mode of division at this stage results in the formation of two pear-shaped forms, but differs from that in the early stages of the disease. Starting again from the round binucleate form, either the large or the small nucleus divides, together with the line; the small nucleus moves to the edge of the parasite, and the chromatic line becomes fainter, and in many instances finally disappears (figs. 45-52). The divided large nuclei frequently remain connected (fig. 46). At this stage one or two vacuoles appear about the middle of the cell, and increase in size. Often the two parasites are connected by three fine protoplasmic strands, two peripheral and one across the middle, a large and small nucleus in each half. First the central connecting strand breaks, and the separation of the two parasites becomes more pronounced, until they are only connected by one strand at their apices. Whilst the pear-shaped forms are still connected in the above described manner, the connecting cytoplasmic

strand may be seen considerably thickened at the middle (fig. 19). This connection becomes smaller and smaller until both parasites separate into two pear-shaped forms; the small nucleus dividing again even before the separation is complete. On the other hand division of the large nucleus may set in first, accompanied or not, by a division of the connecting line (figs. 44-52).

While the pear-shaped forms are still connected, a second small nucleus may arise from the large one (fig. 53 n_2). We have not been able to explain the meaning of this process.

A striking feature of the present stage of the infection is the occurrence of unequal divisions of the parasite. The nuclei of the cells divide in the *usual* way, but the cytoplasm divides into two unequal parts, the smaller parasite assuming a crescent shape. This division may be compared with the sickle-shaped detachment of the cytoplasm described above.

Leishman and Statham[11] describe a similar process in *Leishmania donovani* (*Piroplasma donovani*), with the important difference, however, that eventually nuclei were seen in these detached parts of the parasite.

We, however, could not follow an analogous procedure in *Piroplasma canis* To our minds there are two distinct processes. Either the cytoplasm becomes detached in a regular way without co-operation of the nuclei; or, the nuclei take part in the division. The enucleated particles of cytoplasm probably degenerate, and give rise to the appearance of irregular dark staining masses in the protoplasm of the infected red cells, or the detached part contains one or two nuclei and gives rise to a new parasite (figs. 28 and 29). Rarely chromatin appears to be given off from the nucleus, and become free in the red cell (fig. 30). This process has already been described by Nuttall and Graham-Smith,[18] but its significance is unknown.

The parasites occurring in the blood of organs do not differ markedly from those found in the peripheral and heart blood. As division appears to proceed more rapidly in the organ blood, the parasites are usually slightly smaller and more compact. The free forms, which occur in greater numbers in the organs, divide in the same way as the intra-cellular forms, i.e., round and pear-shaped division. (Compare fig. 43.)

FLAGELLATED FORMS

Flagella-like processes in different species of *Piroplasma* in blood have been frequently described. Bowhill and Le Doux,[2] Nuttall and Graham-Smith,[18] and Kinoshita,[7] describe their occurrence in *Piroplasma canis*; Lignières[12] and Bowhill[1] in cattle piroplasmosis; Fantham[5] in *Piroplasma muris*. These processes have been more frequently observed in cultivation forms, and in developmental forms in the tick, by Koch,[9] Kleine,[8] Kinoshita,[7] and Miyajima.[14]

The meaning of some of these forms has been explained in different ways. Doflein,[4] Nuttall and Graham-Smith,[17] and Hartmann[6] discuss the probability of their being mikrogametes analogous to the mikrogametes of the life cycle in malaria, but nothing in the nature of a proof of this conception has hitherto been brought forward. When we consider the active amoeboid movement of the young parasites, it would certainly appear that most of the flagella-like processes seen must be regarded simply as fine pseudopodia. Kinoshita,[7] on the other hand (figs. 41 and 46), figures a flagellum which arises from a blepharoplast and takes a chromatic stain in the same way as do trypanosome flagella.

Now and again, long flagella-like processes, which were evidently pseudopodia, have been seen in intra-corpuscular forms (figs. 31, 32). (Compare Kinoshita, fig. 9.)

Very rarely true small flagellate forms were seen, especially in blood from the lung; but we were never able to trace the origin of the single flagellum (fig. 27).

Large flagellated forms have been described by Miyajima[14] in cultures of *Piroplasma parvum*, and these forms he describes as intermediate stages in the development of trypanosomes from a typical *Piroplasma*. He discusses at length the possibility of a mixed infection of piroplasmosis and trypanosomiasis of the blood used for his culture experiments, but the facts he brings forward seem to be very much against such a possibility.

Kossel's and Weber's observation, as quoted by Schaudinn,[21] seems to have anticipated Miyajima's observation with regard to large flagellated forms, with the difference that they observed his culture forms in freshly drawn blood.

Nuttall and Graham-Smith[17] in 1905 were the first to describe a

few large forms of *Piroplasma canis*, which simulate the crescents of aestivo-autumnal malaria, having the chromatin sometimes concentrated in the middle, sometimes forming a loose mesh work. These parasites were 10·4 to 10·7 μ long and 1·4 to 1·7 μ broad. They regarded them at first as gametes, but in their last paper they do not consider them to have any connection with *Piroplasma canis*. Their chief reason was the fact that they only found seven 'gametoid bodies' altogether, and these occurred in one animal. No flagella could be observed.

Kinoshita figures somewhat similar parasites (figs. 47, 48, 49) seen in the heart-blood, pancreas and lung, some hours after death. He refers to his figs. 47 and 48 as conjugation forms, and to fig. 49 as an ookinet (?) in accordance with Schaudinn's ideas.

We have been able to trace the development of large biflagellate forms from the normal intra-cellular parasite. In the films, where large biflagellate forms occurred, along with ordinary intra-cellular parasites, forms were also found in which both nuclei were considerably enlarged, as represented in figs. 54, 55. These bodies increase in size, and the smaller nucleus in the meanwhile divides (fig. 56), often remaining connected with the large one by fine chromatic lines. The subsequent changes vary in details, but on the whole two main forms of development may be followed. On the one hand, an irregular number of small round chromatin masses originate from the large nucleus, frequently remaining connected with it by fine chromatic lines, which eventually disappear (figs. 72, 73). From the fact that these masses often appear double, it seems possible that they may divide (figs. 75, 76). At the same time the appearance of the large nucleus changes; the chromatin becomes aggregated at the centre, and a lightly staining area is left between it and the well-defined nuclear membrane (figs. 60, 72-74). Eventually, two flagella are formed, each of which may end in the neighbourhood of a small chromatic mass, but in some cases the flagella appear to have no definite origin (figs. 74, 77, 78).

A second mode of development takes a somewhat different line. The large nucleus frequently buds off at first a small number of granules, and eventually it seems to throw out the whole of its chromatin in form of a large densely staining mass; figures 62-66 representing different stages of this process. The remainder of the

original nucleus persists as a homogeneous lightly staining mass, which retains its original form and moves to one side of the parasite. At this stage usually one long flagellum arises in the neighbourhood of a small chromatic body very often situated at one end of the parasite (figs. 61-64). Shortly after, a second flagellum is formed, sometimes arising in close vicinity to the origin of the first flagellum, sometimes at some distance (fig. 65).

The above described development is very liable to modifications. Occasionally two large masses of chromatin are thrown out of the nucleus (fig. 68), and at the same time these latter sometimes divide (fig. 71).

Whilst these nuclear changes are taking place, the parasites increase in size, and become elongated. The protoplasm changes its appearance, and becomes very loose and vacuolated. The dimensions of fully developed flagellate forms vary between 6 to 8 μ in length and 2 to 3 μ in width.

These forms have been repeatedly observed by us in very small numbers in the peripheral blood of dogs on the day before death. Only in one animal were they abundant, and only in this case have we been able to follow their development. The blood was taken in the morning of the day before death. Films made actually on the day of death did not show any of these forms, neither in the peripheral nor in the heart blood, only two of these flagellated cells being found in organ films (spleen and bone-marrow).

This observation seems to point to the fact that the biflagellate forms of *Piroplasma canis* represent a very transient stage in its life-history. For this reason, it might have been very easily overlooked. We, however, at present, are not able to form a definite opinion as to the significance of this stage in the life-history of the parasite, especially as the subsequent development of the flagellate forms could not be traced.

Up to the present, no observations, either in culture or in the intermediate host, throw any light upon their meaning. Developmental stages of these flagellate forms in some respects resemble those occurring in the development of the flagellate forms in the cultures of *Leishmania donovani.*

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EXPLANATION OF THE PLATES

All the figures are drawn with a Zeiss apochromatic 2 mm. oil immersion lens, aperture 1·40. Oc. 18.

Plate VI

Figs. 1-16.—Breinl's stain.

Fig. 1.—Early amoeboid form.

Figs. 2-4.—Division stages of amoeboid forms.

Fig. 5.—Binucleate amoeboid form.

Figs. 6-9.—Pear-shaped division of binucleate form.

Figs. 10-12.—Stages in the formation of a single bud.

Figs. 13-16.—Stages in the formation of two buds.

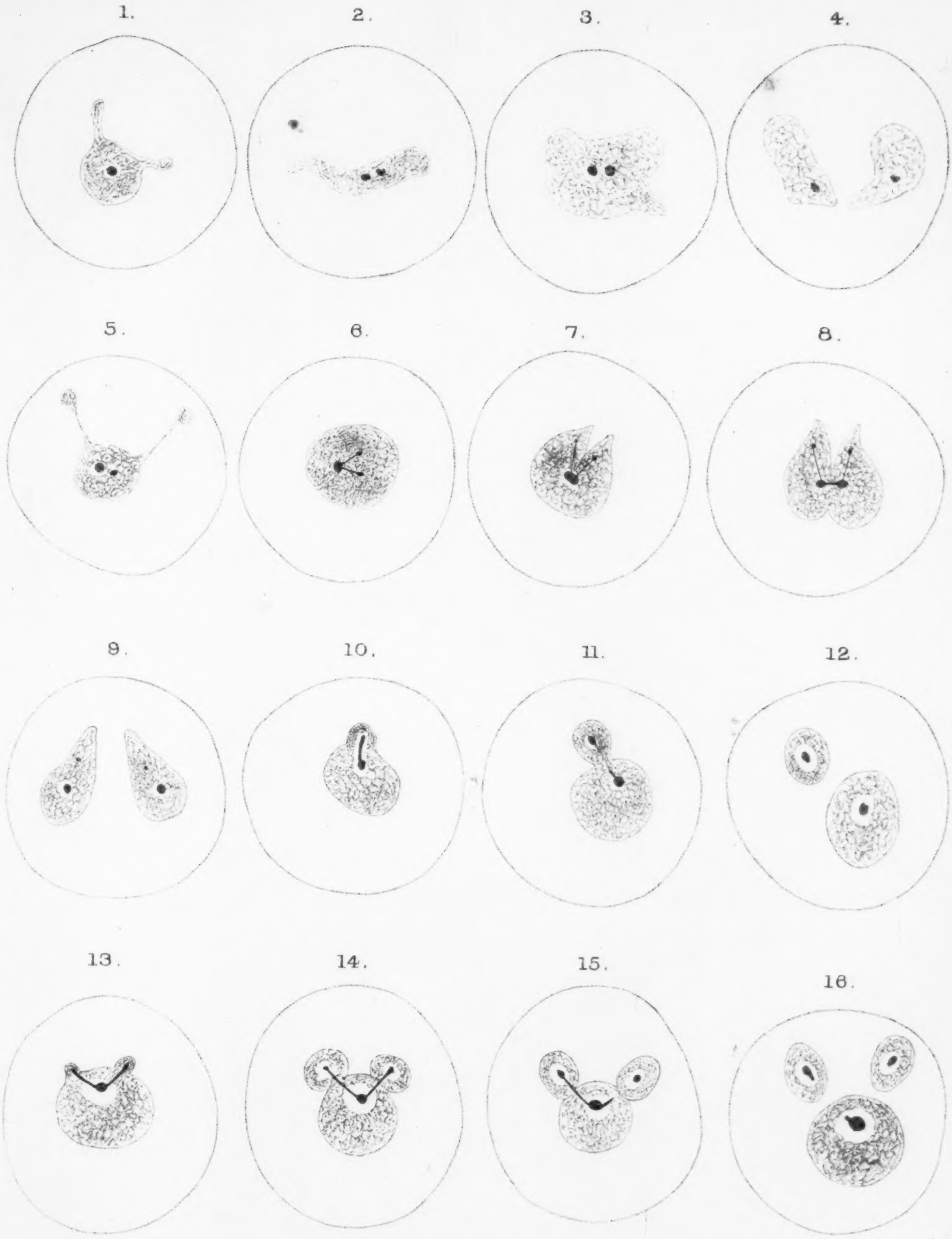

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PLATE VII

Figs. 17-27.—Breinl's stain.

Figs. 28-32.—Heidenhain-Breinl's stain.

Figs. 17, 18.—Different stages of the formation of the small nucleus.

Fig. 19.—Pear-shaped division form, showing thickening of the connecting line.

Fig. 20.—Pear-shaped division; division of small nuclei before separation.

Fig. 21.—Signet ring form, with divided nucleus.

Figs. 22-26.—Formation of the sickle-shaped mass of cytoplasm.

Fig. 22.—Appearance of vacuole at the edge of the cell.

Figs. 23, 24.—Growth of the vacuole.

Figs. 25, 26—Separation of the sickle-shaped cytoplasmic part.

Fig. 27.—(*a*) Free pear-shaped binuclear form.
(*b*) Free small flagellate parasite.

Figs. 28, 29.—Unequal division.

Fig. 30.—Extrusion of chromatin into the red cell.

Fig. 31.—Amoeboid form with long pseudopodium.

Fig. 32.—Division of same.

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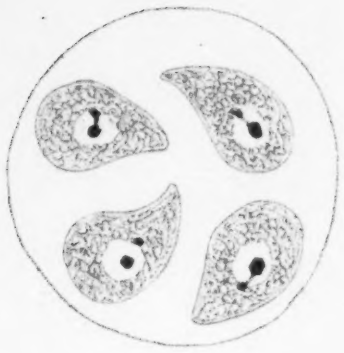

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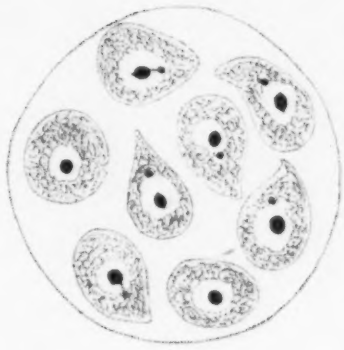

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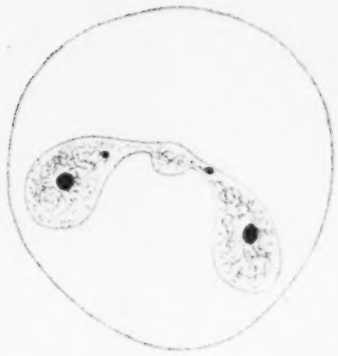

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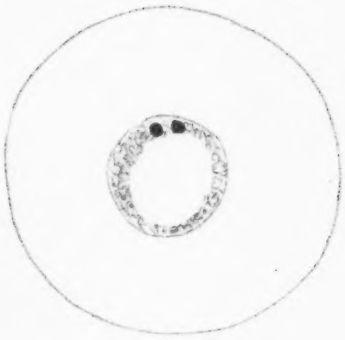

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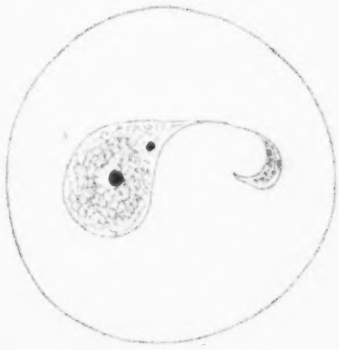

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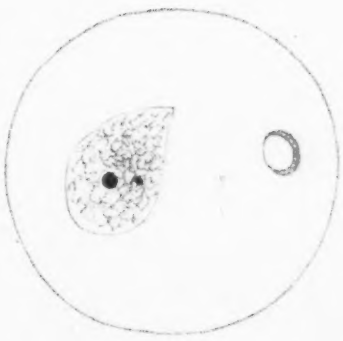

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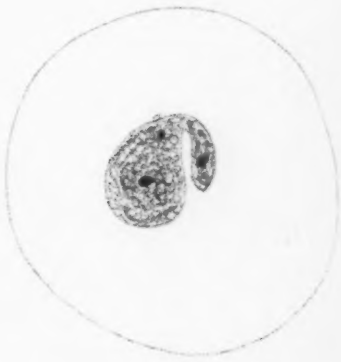

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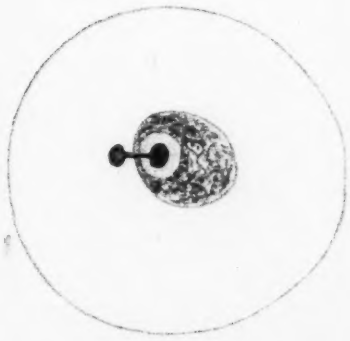

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PLATE VIII

Figs. 35-48.—Heidenhain's stain. Later stages of division.

Figs. 33-40.—Division of round forms.

Fig. 41.—Round intra-cellular binucleate form.

Fig. 42.—Formation of small nucleus.

Fig. 43.—(*a-c*) Division of free round forms. (*d*) Formation of small nucleus in free form.

Figs. 44-48.—Stages in the late pear-shaped division.

Fig. 44.—Division of large nucleus and chromatic line

Fig. 45.—In the left parasite division of small nucleus. In right parasite appearance of vacuole and commencement of division of large nucleus.

Fig. 46.—Both nuclei divided; daughter cells connected by three strands.

Fig. 47.—Disappearance of middle connecting strand.

Fig. 48.—Rupture of lower connecting strand.

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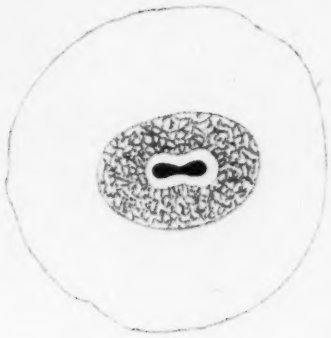

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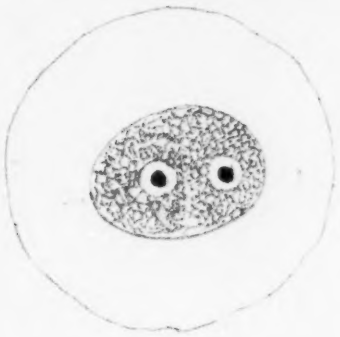

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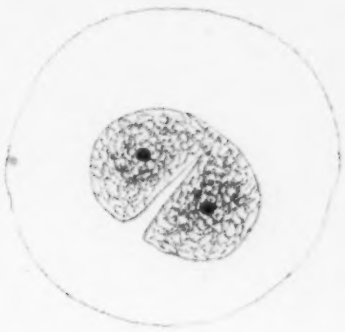

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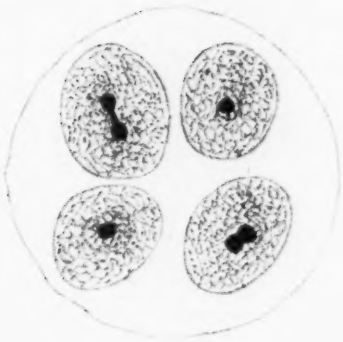

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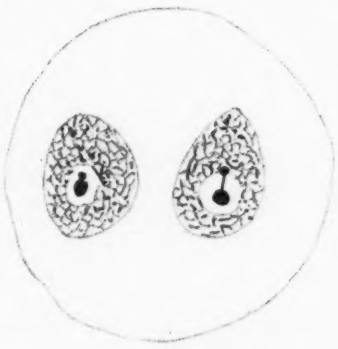

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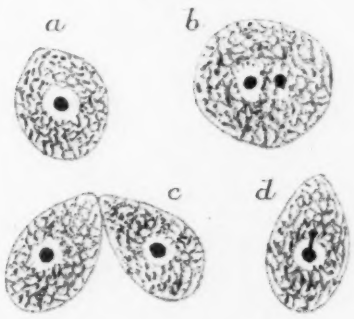

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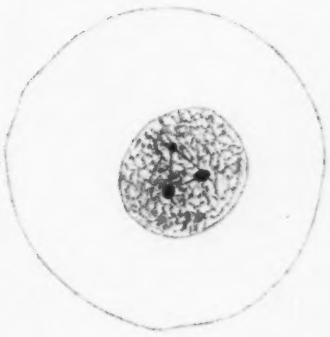

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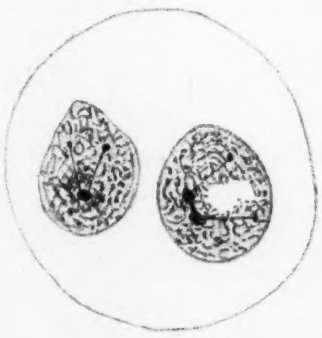

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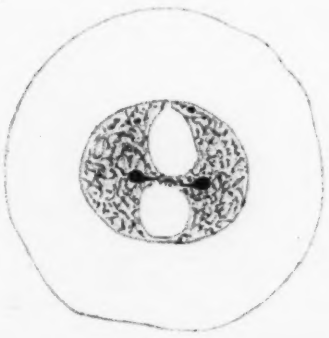

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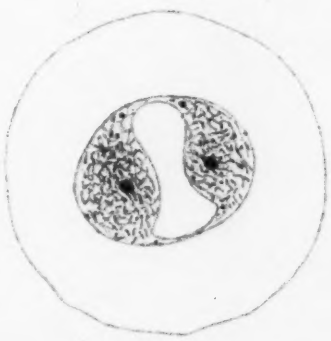

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Plate IX

Figs. 49-78.—Heidenhain-Breinl's stain.

Figs. 49-50.—End stages of pear-shaped division.

Fig. 51.—Division of small nuclei before separation of daughter cells.

Fig. 52.—Division of large nuclei before separation.

Fig. 53.—Formation of a second small nucleus (*n*).

Figs. 54-78.—Formation of large biflagellate parasites.

Figs. 54, 55.—Swelling up of nuclei, in intra-cellular parasites.

Fig. 56.—Division of enlarged small nucleus.

Fig. 57.—Extrusion of chromatin from the nucleus.

Figs. 58, 59.—Swelling up of nucleus in three parasites.

Fig. 60.—Transformation of large nucleus.

Fig. 61.—Formation of flagella.

Figs. 62-64.—Stages in the extrusion of chromatin from the nucleus.

Fig. 65.—Formation of second flagellum.

Figs. 66, 67 and 69.—Large elongated biflagellate forms.

Fig. 68.—Extrusion of two masses of chromatin from the nucleus.

Fig. 70.—Large flagellate form after extrusion of chromatin, containing a number of large granules.

Fig. 71.—Division of extruded chromatin and disappearance of the remains of the original nucleus.

Figs. 72-78.—Stages in the development of large biflagellate forms with characteristic nuclei.

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COMPARATIVE CHEMO-THERAPEUTICAL STUDY OF ATOXYL AND TRYPANOCIDES

PART I

BY

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(*From the Runcorn Research Laboratories*)

(*Received for publication* 14 *May*, 1908)

Atoxyl, sodium-p-amino phenyl-arsenate was introduced in the treatment of Trypanosomiasis by Thomas and Breinl[1] (1905), and its specific therapeutic value for sleeping sickness has been more or less recognised. It contains from 25·95 to 20·78 per cent. of arsenic; the difference depends on the water of crystallisation, as shown by Moore, Nierenstein and Todd,[2] Ehrlich and Bertheim,[3] and others.

Arsenic in the form of Atoxyl is much better tolerated by the animal organism than in the form of sodium arsenate; the therapeutic value of the Atoxyl, therefore, was attributed to the fact that much more arsenic could be administered in this new form. It was supposed to act simply as an internal *antiseptic*, and was thought to kill the parasites in direct proportion to the amount of arsenic introduced.

Some experiments made in June, 1907, by Breinl and Nierenstein seemed to disprove this idea. In an attempt to produce an active immunity against Ngana, mixtures of Atoxyl and trypanosomes were injected in different proportions, and after different periods of contact, with the idea that by increasing the amount of trypanosome-infected blood and decreasing the amount of Atoxyl, and by lessening the time of contact, a point might be reached at which virulent trypanosomes could be injected with impunity.

The results obtained, however, were not what were expected; dogs, rabbits and donkeys were used for the experiments, but invariably after the first injection, even after exposure of the mixture for forty-five minutes to a temperature of 37° C., the animals became infected after a normal incubation period. This fact seemed to suggest that the action of Atoxyl was not simply disinfectant, but was the result of a co-operation between the living tissues and the drug.

Uhlenhuth, Hübner and Woithe[4] in their experimental study of the action of Atoxyl on *T. equiperdum* came to a similar conclusion. They state (p. 296):—

'Unsere Meinung geht jedenfalls dahin, dass der Chemismus der Atoxylwirkung kein so einfacher ist, wie ihn die Theorie der Arsenspaltung supponiert, das vielmehr beim Zustandekommen des wunderbaren therapeutischen Effektes die Körperzelle eine ganz hervorragende Rolle spielt.'

This observation of ours, confirmed by Uhlenhuth, Hübner and Woithe, was the starting point for the following study of the therapeutical action of Atoxyl.

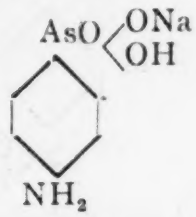
The experiments were divided into two groups, the action of Atoxyl and similar compounds on serum proteids being studied *in vitro* and *in vivo*, respectively. Only the results of the first series are here recorded.

Technique.—20 c.c. of normal serum and 20 c.c. of a 2 per cent. solution of the compound were shaken up for twenty-four hours, and the proteids afterwards were precipitated with 35 c.c. of a 2 per cent. solution of tannic acid. The precipitate was then carefully washed for about forty-eight hours and arsenic estimations of the filtrate were made from time to time until no trace of arsenic could be found in the filtrate. The precipitate was treated with 10-15 c.c. of concentrated sulphuric acid, and digested in a Kjeldahl flask in the usual way.

The arsenic estimations were made by Sanger's[5] method. Instead of hydrochloric acid, gold chloride was used as a developer, and proved much more sensitive.

In those cases in which arsenic was found in the precipitate after digestion, some of the original product was dialysed against water in a parchment sausage-skin, and the dialysate was evaporated to dryness and tested for arsenic.

1. Atoxyl and Serum

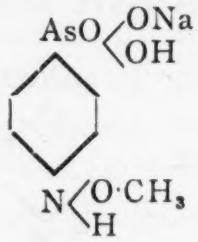
Atoxyl = 

Serum Used.	Arsenic Estimation in Precipitate.	Arsenic Estimation in Dialysate.
Horse serum	arsenic present	—
Horse serum	arsenic present	arsenic absent
Horse serum	arsenic present	arsenic absent
Goat serum	arsenic present	arsenic absent
Goat serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent

2. Sodium Arsenate and Serum

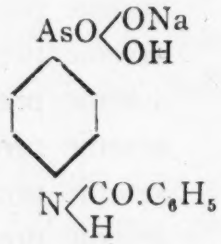
Serum Used.	Arsenic Estimation in Precipitate.	Arsenic Estimation in Dialysate.
Horse serum	arsenic absent	—
Horse serum	arsenic absent	—
Horse serum	arsenic absent	—
Goat serum	arsenic absent	—
Goat serum	arsenic present	arsenic present
Goat serum	arsenic absent	—
Goat serum	arsenic absent	—
Donkey serum	arsenic absent	—
Donkey serum	arsenic absent	—
Donkey serum	arsenic absent	—
Donkey serum	arsenic absent	—
Donkey serum	arsenic absent	—

3. Acetylated Atoxyl and Serum.

Mono-acetylated atoxyl = 

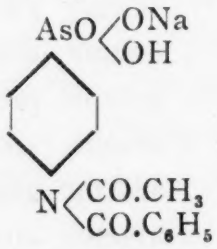
Serum Used.	Arsenic Estimation in Precipitate.	Arsenic Estimation in Dialysate.
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent (?)
Donkey serum	arsenic present	arsenic absent

4. Benzoylated Atoxyl and Serum

Mono-benzoylated atoxyl = 

Serum Used.	Arsenic Estimation in Precipitate.	Arsenic Estimation in Dialysate.
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent

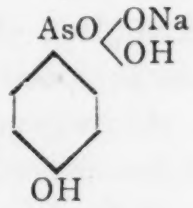
5. Benzoyl-acetyl-atoxyl and Serum

Benzoyl-acetyl-atoxyl = 

Serum Used.	Arsenic Estimation in Precipitate.
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent

6. SODIUM-p-HYDROXYL-PHENYL-ARSENATE* AND SERUM

Sodium-p-hydroxyl-phenyl-arsenate =



SERUM USED.	ARSENIC ESTIMATION IN PRECIPITATE.
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent

It is evident from the foregoing experiments that a combination takes place respectively between proteins and Atoxyl, mono-acetylated Atoxyl, and mono-benzoylated Atoxyl; whilst no combination occurs respectively between these proteins and sodium arsenate, acetyl-benzoyl Atoxyl, and sodium-p-hydroxy-phenyl-arsenate.

It might be mentioned that there is a considerable difference in the results obtained by the treatment of Trypanosomiasis by means of the above-mentioned compounds. Whereas Atoxyl and mono-acetylated Atoxyl act promptly on the parasites, the effect of sodium arsenate is less pronounced, that of sodium-p-hydroxy-phenyl-arsenate is nil.

The analogy between the way in which these compounds behave with proteins, and their action on trypanosomes, is very suggestive. We are, hence, led to believe that this combination with the proteins is of importance in trypanocidal drugs, and have now to consider how Atoxyl and its derivatives become attached to the proteins.

Ehrlich[6] has compared the action of a drug to that of a dye. We know that it is necessary for a dye to possess a chromophoric group—a chemical radical which causes it to be coloured—and a chromogenic

* Our thanks are due to Messrs. Burroughs, Wellcome & Co., who kindly supplied this drug.

group, which renders it a dye. This is easily illustrated by the following example :—

Azo-benzene ($C_6H_5N=NC_6H_5$), which contains the chromophor $N=N$, is coloured, but does not possess dyeing properties. It only becomes a dye when the chromogenic group OH or NH_2 enters. Similarly, for example, oxyazo-benzene ($OH.C_6H_4N=NC_6H_5$) and amino-azobenzene ($H_2N.C_6H_4N=NC_6H_5$) are dyes. Their dyeing value increases with the number of chromogenic groups introduced. For this reason tri-amino-benzene ($NH_2.C_6H_5N=N.C_6H_3(NH_2)_2$) is a much better dye than amino-azo-benzene.

When we apply the same theory to the therapeutics of Atoxyl, we find that sodium-phenyl-arsenate $\left(C_6H_5AsO\left\langle\begin{matrix}ONa\\OH\end{matrix}\right.\right)$ (which has been proved by Plimmer and Thomson[7], and also in this laboratory, not to possess any curative effect), and also sodium-p-hydroxy-phenyl-arsenate $\left(OH.C_6H_4AsO\left\langle\begin{matrix}ONa\\OH\end{matrix}\right.\right)$ do not combine with the proteins, whilst atoxyl $\left(NH_2C_6H_4AsO\left\langle\begin{matrix}ONa\\OH\end{matrix}\right.\right)$ combines with the proteins and acts on trypanosomes; mono-acetylated atoxyl $\left(CH_3CONH.C_6H_5AsO\left\langle\begin{matrix}ONa\\OH\end{matrix}\right.\right)$ combines and is curative, while fully acetylated and benzoylated atoxyl $\left(\begin{matrix}CH_3CO\\C_6H_5CO\end{matrix}\right\rangle N.C_6H_5AsO\left\langle\begin{matrix}ONa\\OH\end{matrix}\right.\right)$ does neither.

Hence, we suggest that in Atoxyl the amido group (NH_2-group) and in mono-acetylated Atoxyl the imido group (NH-group) play the same rôle as the chromogenic group in a dye. It has already been pointed out that the action of Atoxyl has generally been explained as being due to the arsenic, and the advantage of its use is that more arsenic could be introduced in the organism in form of Atoxyl than in form of sodium arsenate; it might be argued from this point of view that the action of Atoxyl is as follows :—

The Atoxyl attaches itself to the proteins; the benzene nucleus is slowly oxydised by the tissues and the arsenic is set free; so that,

when combined with the tissues, Atoxyl acts as a storage for effective arsenic.

This, however, is apparently not the case. It is well known that Trypanred, Afridol blue and Afridol violet, also Parafuchsin, have an effect on trypanosomes comparable to that of Atoxyl. These compounds do not contain arsenic, but a large number of amido groups. Further, Laveran,[8] also Thomas and Breinl, have found that sodium arsenate in combination with trypanred acts much better than sodium arsenate alone.

We have, therefore, reason to believe that the amido group in Atoxyl, and in the above-mentioned colouring matters, has a specific action on trypanosomes, and that in Atoxyl the effective part is not only the arsenic, but also the amido group.

How this group acts on the parasites is engaging our attention at present, and will form the subject of a subsequent communication.

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ON THREE NEW SPECIES OF CULEX COLLECTED DURING THE ANTI-MALARIAL CAMPAIGN IN MAURITIUS IN 1908*

BY
D'EMMEREZ DE CHARMOY

(WITH ONE PLATE)

(*Received for publication* 12 *April*, 1908)

Culex arboricollis, n. sp.

MALE.—*Head:* eyes greenish; occiput yellowish, with long, white and yellow, curved scales and a few hair-like black scales; the yellow scales are placed closely in the line separating the eyes. The *antennae* bear long hairs which are pale yellowish apically and greyish-black basally; the segments of the basal half furnished with very long, narrow curved scales;† apical segment with a few short hairs; the basal segment with short, flat, white scales. Palpi of four segments, as long as the proboscis, with narrow white bands at the base and the apex of the second, third and fourth segments; white scales are desseminated over all the segments. Proboscis black, with the apex paler and a yellowish band in the middle.

Thorax black, covered sparsely with long, narrow curved, white and golden scales, and long, black, hair-like scales; those portions of the thorax which are not covered with scales form velvet black spots. Scutellum bordered with flat whitish scales, and dark hair-like scales; metanotum nude, black.

Abdomen velvety black, with whitish basal bands; apical segment with a few whitish scales at apex; all the segments with long yellowish marginal hairs.

Legs black, with more or less loosely scattered, yellowish scales; the articulations of the femora and tibiae are basally and apically banded; the tarsi are black without any coloured scales; metatarsi of front legs are basally banded; the other tarsal segments are black; in the mid leg the metatarsi and the first tarsal segments are basally

* During the Expedition of Professor Ross, F.R.S., C.B.

† This remarkable insect bears some resemblance to Theobald's genus *Lophoceratomyia*, though it is quite distinct, and a new genus will probably be erected to receive it.—R. NEWSTEAD.

banded; in the hind legs the metatarsi are basally banded with yellowish scales, and apically with white scales; all the remaining segments are basally and apically banded with white scales.

Wings spotted. The black spots on the costa extend to the auxiliary vein. They are seven in number and are situated as follows: two small basal ones, the second a little larger than the first, the third one having a white dot in its middle, the fourth and fifth ones united on the auxiliary vein by black scales, the sixth placed obliquely, the seventh near the apex; the other veins are irregularly spotted with white scales, the last vein* which bears the black fringe is regularly spotted white and black on its basal half. The underside of the body presents the following markings: The *pleura* densely covered with imbricated, flat, whitish scales; the trochantae, coxae, and the base of the femora are covered with white scales; the ventral segments of the abdomen are spotted basally with white scales and apically with a well-defined, narrow, white line.

FEMALE.—Proboscis black, with a few scattered white scales, and a white band just below the first anterior third. Palpi longer than the half of the proboscis, with a few scattered white scales and white bands; the apical segment bears two moderately long hairs. The fore part of the occiput is covered with long, narrow curved, white scales; the hind portion with yellow, upright forked scales; the anterior lateral portions with black, upright forked scales. Scutellum with a median and two lateral tufts of long, black hairs, and a few long, flat, curved white scales. The thorax and pleurae as in the male. Halteres yellowish, with small, white scales. The larvae of this species were found by Professor Ronald Ross in the holes of trees at Vacoas; and although the larval habitat was situate near dwellings, no adults were seen in houses or verandahs. This well-marked species is apparently uncommon, and comes near *Culex mimeticus*.

Culex fowleri, n. sp.

FEMALE.—*Proboscis* brown, base paler, with whitish scales. *Palpi* black, with a few long, black hairs; the apex white. *Antennae* brown, spotted with white; first segment bearing white scales. Occiput at the sides covered with flat, imbricated, white and black

* ? Costa.—R. N.

scales; the median portion covered with long, white, narrow, curved scales, black, upright forked scales, and black hair-like scales.

Thorax brown, with two sub-median greyish lines, with long narrow curved, golden scales and black hair-like scales. Scutellum with white scales and golden, hair-like scales.

Abdomen black, with white basal bands. First abdominal segment with a basal white dot and apical white line, the other segments with apical white bands; the penultimate one with two apical spots; the last with lateral white spots. The underside of the abdomen with basal and apical bands.

Legs. Under surface of the femora and trochantae of the posterior legs white; the upper surface brown, with small scattered spots; femora of the fore legs with white scales and hairs at their apices. Metatarsi with the first and second segments white at the base; the femora and tibiae of the mid legs are marked with white at their basal and apical parts. Metatarsi with the first and second segments white at the base. The femora of the hind legs white apically. The veins of the *wings* are covered with brown and white scales. Male similar.

This species is easily distinguished from all other members of the genus *Culex* by the black and white spots on the body of the insect. It was discovered by Major P. Fowler at Vacoa.

Culex ronaldi, n. sp.

FEMALE.—*Proboscis* brown, with a yellowish median band. *Palpi* brown, bearing long hairs. *Antennae* brown; auxiliary hairs black, longer than those situated on the segments which are whitish. Eyes black. Occiput bearing scattered, white, long, narrow curved scales which form a continuous white line round the eyes. Upright forked scales black; hair-like scales black. Thorax brown, covered with long, narrow curved, golden scales, and long, black, hair-like scales, these are numerous on the posterior lateral margins. Scutellum with long, narrow, golden scales and black hair-like scales. Halteres white, yellowish at the tips. *Abdomen* black, with basal white bands. Scales of the wings brown. *Legs*: The femora are white apically; the tibiae white basally. Tarsi with narrow dusky white basal bands. Under side of trochantae and femora covered with white scales.

MALE.—*Palpi* a little longer than the proboscis, brownish and hairy at the apex, with a white band at the base of the second segment and in the middle. The articulation of the segments of the antennae are black, the remaining portions of the segments whitish. Eyes black. The lateral portions of the head white; median portion with long, narrow, curved, white scales; upright, forked and hair-like scales, black. The under side of the abdominal segments with large lateral white bands, the penultimate one descending obliquely to the lateral margins.

Found in the larval stage by Major P. Fowler in the broad moat outside Fanfava Bastian, in December, 1907, and January, 1908. Ground marshy, water from few inches to one foot deep, with much coarse grass. The larvae occurred in association with numbers of *P. costalis.*

LIST OF CULICIDAE OF MAURITIUS

ANOPHELINAE (Anophelines)

1. *Pyretophorus costalis*, Loew (1866).
   Anopheles costalis, Loew (1866).
   A. gambiae, Giles (1902).
   A. gracilis, Dönitz (1902).

This species has been proved to be the principal carrier of Malaria at Phœnix and Vacoa where they are most numerous. Daruty and d'Emmerez found it very common at Port Louis in 1900. In some places near the sea shore it is uncommon, for example at Rre. At Seche and Maheburgh very few have been found.

2. *Myzorhynchus mauritianus*, d'Emmerez and Daruty (1900).
   Anopheles paludis var. *similis*, Theobald (1901).
   A. tenebrosus, Dönitz (1902).

Very common everywhere and especially at Curepipe, Vacoa and Phœnix. All the specimens caught in the open air at Phœnix, Vacoa, where malaria is prevalent, were found not to be infected.

3. *Nyssorhynchus maculipalpis* (Giles).
   Anopheles maculipalpis, Giles (1902).

Not common; a few specimens only were caught by Major Fowler at Iron Fanfaren in Port Louis.

CULICINAE

4. *Stegomyia scutellaris*† (Walker) (1859).
Culex scutellaris, Walker.
C. albopictus, Skuse.
C. variegatus, Doleschard.

Very common everywhere, certainly the most abundant species of the island; the larvae occurred in tins, leaves, holes in trees and in the Ananas Sauvages.

5. *Stegomyia fasciata*, Fabricius (1805).
Culex fasciatus, Fabricius (1805).
C. calopus, Meigen (1818).
C. taeniatus, Wiedemann (1898).
C. elegans, Ficalbi (1896).
C. rossii, Giles (1899).
C. exagitans, Walker (1856).
C. konuoupi, Brullé (1832).
C. zonatipes, Walker.
C. formosus, Walker (1848).
C. frater, Robineau-Desvoidy (1887).
C. excitans, Walker (1848).
C. viridifrons, Walker (1848).
C. inexorabilis, Walker.
C. bancrofti, Skuse (1886).
C. mosquito, Aribalzaga (1891).
C. annulitarsis, Macquart (1848).
C. impatabilis, Walker (1860).

Very common near the sea shore, in Port Louis; but rather scarce in the high parts of the islands.

6. *Culex arboricollis*, n. sp., d'Emmerez de Charmoy (1908).

The larvae of this interesting species were found in the holes of trees at Vacoa. It is, however, very scarce.

7. *Culex ronaldi*, n. sp., d'Emmerez de Charmoy (1908).

Not common, the larvae were found at Iron Fanfaron. The larvae can be easily differentiated from those of the other species of this Island by its very long siphon tube.

† Theobald (Genera Insectorum, p. 19, 1905) gives *Scutomyia notoscripta* (Skuse) priority.—R.N.

8. *Culex annulioris*, Theobald (1901).

Only one specimen of this species was taken by Colonel Peterkin, at Vacoa.

9. *Culex fowleri*, n. sp., d'Emmerez de Charmoy (1908).

Not common. A few specimens obtained from larvae caught by Major P. Fowler.

10. *Culex tigripes*, d'Emmerez and Daruty (1900).
Culex maculicrura, Theobald (1901).

Very common, and one of the largest species known. The larvae are carnivorous and they also eat each other.

11. *Culex fatigans*, Wiedemann.
Culex anxifer, Coquerel (Bigot).

The commonest of all the species. It is very numerous all over the island and very troublesome during the night. The larva are to be seen in all artificial collections of water.

12. *Culex*, spec. incert (male).

A single specimen, caught by Major Fowler agrees in some respect with *C. annulioris*; but it is evidently distinct, though not sufficiently well preserved to render identification possible.

ANOPHELINES THAT TRANSMIT MALARIA

Pyretophorus costalis

228 examples were caught at Clairfond Marsh between February 4, 1908, and February 20, 1908. 73 of these were examined, of which 10 were infected (i.e. 13·7 per cent.).

? *Myzorhynchus mauritianus*

54 examples which were fed on blood containing crescents and other gametes gave one positive result. (Round pigmented cells ? dead zygotes eight days after the first meal.) 56 other examples caught wild were negative.

EXPLANATION OF PLATE X

Culex tigripes

Fig. 1.—Right ventral half of the head of the larva.

Fig. 2.—Anal segments of the larva with siphon tube.

Fig. 3.—Labial plate of the larva.

Culex ronaldi, n. sp. (page 259)

Fig. 4.—Anal segments of the larva with siphon tube.

Fig. 5.—Antenna of the larva.

Culex arboricollis, n. sp. (page 257)

Fig. 6.—Wing of the male.

Fig. 7, *a*, and *b*.—Claws of the tarsi.

Fig. 8.—Proboscis and antenna of the female.

Culex fowleri, n. sp. (page 258)

Fig. 9.—Portion of the wing shewing distribution of the scales.

Fig. 10.—Wing scales.

Nyssorhynchus maculipalpis

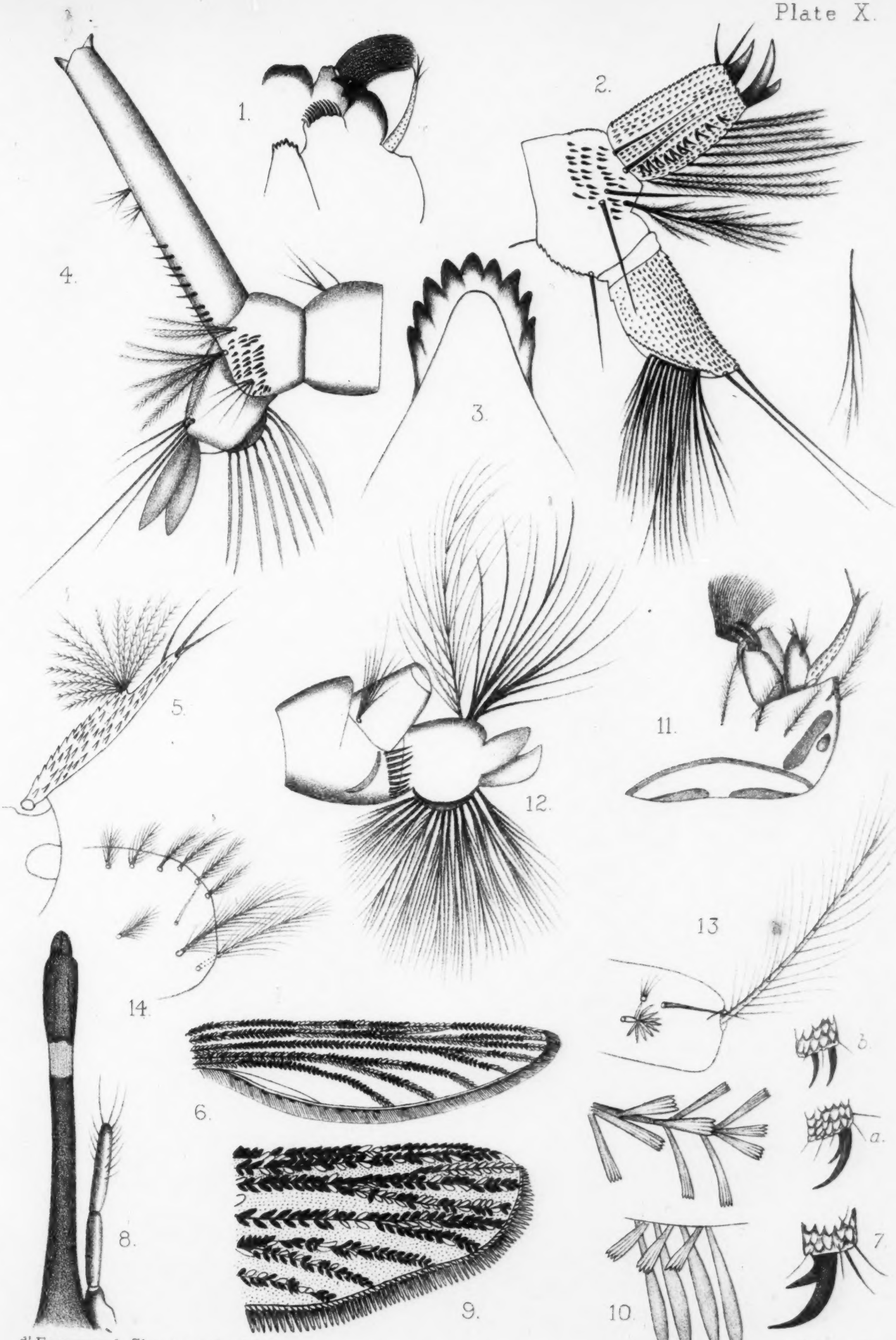
Fig. 11.—Right dorsal portion of the head of the larva.

Fig. 12.—Anal segments of the larva.

Fig. 13.—Second anterior abdominal segment of the larva shewing palmate and marginal hairs.

Fig. 14.—Right anterior portion of the thorax of the larva.

Plate X.



d' Emmerez de Charmoy ad nat. del.

W. Mills, Lith.

CONCERNING THE TREATMENT OF EXPERIMENTAL TRYPANOSOMIASIS*

PART II

BY

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(*Received for publication* 14 *July*, 1908)

INDEX

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Our thanks are also due to Professor Ehrlich for acetylated Atoxyl (Acetyl-para-amino-phenyl-arsenic acid, 8 and 11); and to Messrs. Bayer and Co., Elberfeld, Germany, for the various colouring matters on which we report in Section IV.

I. INTRODUCTION

This paper is a continuation of the experimental work on the treatment of experimental trypanosomiasis already published by us.[1, 2]

The technique of the present series of experiments was the same as employed in our former work. *Trypanosoma brucei* was again used in all of our experiments, unless it is otherwise specified, because of its great virulence for experimental animals, which makes it possible for conclusions to be drawn more quickly and more certainly from work done with it than is the case with any other pathogenic trypanosome.

The strain with which we worked killed untreated rats in from three to five or seven days. In each series of experiments described, animals of about equal body weight were used as far as possible. All inoculations were made subcutaneously with blood mixed with Sodium citrate solution in saline, and approximately equal quantities of blood were used in every inoculation of animals of the same species where results were required for the comparison of different drugs. The usual quantity of infecting blood inoculated varied from 1 c.cm. to 5 c.cm. for rats to from 2 c.cm. to 5 c.cm. for guinea pigs and as much as 10 c.cm. for donkeys. The strain which was used for infecting purposes was always kept going in untreated animals, and therefore there is no possibility of a resistance to drugs, acquired by the trypanosomes, having militated against the success of our experiments.

Treatment was commenced in no instance before there were definite signs of a well-established infection, such as the constant presence of the parasites in the peripheral circulation of the infected animal.[12] It may be noted here that, however valuable they may be as an indication of the trypanocidal value of the drugs used, experiments, in which treatment is commenced on the first or second day of the appearance of the trypanosomes in the blood, and therefore, before the infection is well-established, are, for practical purposes, valueless. Because, to give but one reason, it would certainly, as a rule, be practically impossible to treat a naturally infected animal at such an early stage of the disease. We suspect that early infections are so much the more easily treated not only

because there are fewer parasites present, but also because developmental, resistant forms have not yet been produced; we also suspect that it is to such resistant forms that recurrences are often due.[1, 2, 9]

Control animals of the same species which remained untreated were inoculated at the same time as the treated animals in every instance where the therapeutic effect of any drug was tried; and in experiments with *Trypanosoma gambiense*, additional controls were inoculated from the experimental animal just before its treatment was commenced, to definitely prove for each experiment the virulence of the infecting parasite.

The Atoxyl used came from the Charlottenburg firm which first manufactured the drug. Unless it is otherwise stated, it was used in a freshly-made five per cent. solution in water previously sterilized at 100°. The Mercury bichloride was usually employed in a one per cent. solution in water. The poisonous dose of each substance for the species of animal employed was always ascertained as a preliminary step to experimentation, and the largest possible therapeutic dose was used in each instance. Save when otherwise stated, all drugs were given subcutaneously.

The routine examination of the blood was made in fresh three-quarter inch square coverslip preparations of blood from the tail or ear according to the animal. The blood of important animals was centrifugalised whenever it seemed necessary, as, for example, when trypanosomes could not be found in the blood by the ordinary examination, although the temperature was elevated. As a rule, for the first ten days or fortnight succeeding completion of the treatment the animals were examined daily. As they lived longer the examinations became less frequent, until they were done approximately weekly or, in the case of experiments made with *Trypanosoma gambiense*, twice weekly. The blood in the less successful experiments, for example, the donkeys, was examined daily during the whole time these animals were under observation. The blood of any animal evidently ill was, of course, immediately examined, and if trypanosomes were not seen, subinoculations were at once made. Subinoculations of considerable quantities of blood were made also at intervals from animals which had been apparently successfully treated. All such subinoculated animals were kept under observation

and examined frequently for at least three months in the case of *Trypanosoma brucei*, longer, if *Trypanosoma gambiense* was in question, before being considered to be uninfected. Rats and mice were, as a rule, used for such subinoculations in preference to other animals, as indeed they were throughout our entire work.

II. COMPLETION OF PART I [1].

The three control rats, treated by Atoxyl alone, which were alive at the date of publication of the last paper, have all since died of trypanosomiasis at periods varying between one hundred and two hundred and twenty-six days after the cessation of treatment.

Trypanosomes (*Trypanosoma brucei*) have never recurred in any of the rats from which they had disappeared through the use of Atoxyl followed by bichloride of Mercury. All of the rats, save one, have died of pneumonia or have been killed because of skin diseases. None of the animals subinoculated from them, either before or at death, have become infected. One rat lived five hundred and nineteen days after inoculation, and then died from a skin affection. Nearly all the others had lived well over two hundred days before they died or were killed. It therefore seems justifiable to conclude that the rats, mentioned in this series of experiments, from which the trypanosomes were absent when Part I of this investigation was published, were definitely cured of their infection by *Trypanosoma brucei*.

All the rats treated by Atoxyl followed by Donovan's solution died of pneumonia in from one hundred and thirteen to one hundred and fifty-one days after inoculation; three of them died on the one hundred and fifty-first day. Trypanosomes had reappeared in none of them, nor have they appeared in the animals subinoculated from them. It seems probable that these animals were also cured of their trypanosome infection.

It was shown by the inoculation and normally following death of a series of rats so cured that no immunity was acquired by animals which had recovered from an infection by *Trypanosoma brucei* after treatment with Atoxyl followed by Mercury; [11] neither was immunity conferred upon the young of such animals, for a series of eight young rabbits, both of whose parents had been treated and recovered from a trypanosome infection, became infected and quickly died on inoculation.

III. INEFFICACIOUS SUBSTANCES

Because of the marked action of quinine on other protozoa, it was thought that substances derived from it, or resembling it, might be trypanocidal. Since Atoxyl, an organic arsenic-containing compound, kills trypanosomes, other compounds of like nature were tried alone, or in combination with various drugs; various other substances were tried in the same way. None of them were of value. The following are the drugs and combinations of drugs tried without advantage on rats infected with *Trypanosoma brucei*:—

Quinine.
Cinchonine.
Cupreine.
Quinine-cacodylate.
Quinine-cacodylate followed by Sublimate.
Quinine-cacodylate followed by Iron-cacodylate.
Quinine-cacodylate followed by Iron chloride.
Arrhenal.
Potassium bichromate.

IV. ANILINE COLOURING MATTERS

Our work in this direction is still very incomplete; we give, however, what results we have obtained. We have adopted the following routine method of gaining an idea of the efficiency of an untried substance. A series of rats is inoculated; some are left untreated, others are given one full dose of the substance to be tried. The average difference in days between the date of death of the controls and of the treated animals is noted. Its value is indicated for each of the drugs mentioned in the following list (column 3); our usual virulent strain of *Trypanosoma brucei* was the infecting parasite in each case.

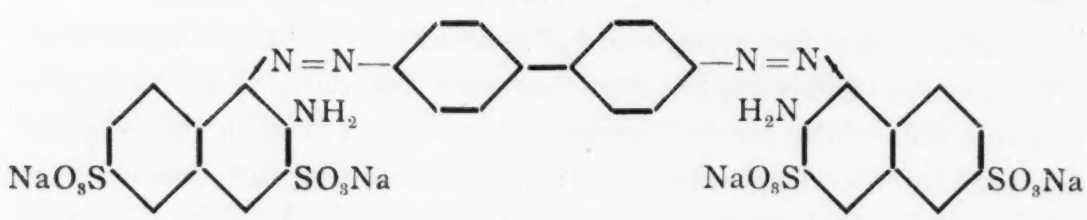
Phenolphthalein	Average of 6 rats	0 days
Acetylated Phenolphthalein	Average of 9 rats	2·3 days
Acetylated Phenolphthalein in alkaline solution	Average of 4 rats	3 days
Methylated Phenolphthalein	Average of 2 rats	0 days
Fluorescein	Average of 4 rats	0 days
Acetylated Fluorescein	Average of 3 rats	1 day

Methylated Fluorescein	Average of 4 rats	0 days
Eosin	Average of 4 rats	1·5 days
Erythrosine	Average of 4 rats	0 days
Floxine	Average of 3 rats	2 days
Bengal rose	Average of 2 rats	0 days
Rhodamine	Average of 3 rats	5·5 days
Diazotized Rhodamine	Average of 4 rats	5 days
Afridol violet	Average of 3 rats	4 days
Afridol blue	Average of 6 rats	5 days
Trypanred	Average of 5 rats	6 days
Acetylated Rhodamine	Average of 2 rats	5 days
Azogreen	Average of 2 rats	1·5 days
Fuchsin	Average of 7 rats	8·5 days
New Green	Average of 6 rats	0 days
Crystal Violet	Average of 6 rats	0 days
Antipyrin	Average of 6 rats	2·5 days

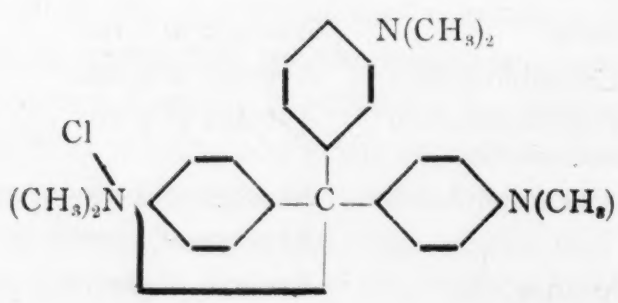
A few experiments were made with Parafuchsin,[3] given subcutaneously; they were far from satisfactory.

Since the discovery of 'Trypanroth,' I, by Ehrlich and Shiga,[4] great attention has been given to the trypanocidal properties of different aniline dyes belonging either to the Diazo-group, the 'Afridols' of Mesnil and Nicolle,[5] or to the Triphenylmethane group, the 'Malachite Green,' II, of Wendelstadt and Fellmer.[6]

I



II



Mesnil and Nicolle, in their excellent work on the action of Benzidines[7] (Diazo-colouring matters), have already pointed out that even the slightest difference in the constitution of a substance may have a great effect upon its trypanocidal action. This point is particularly well illustrated in the two following compounds, of which III has a very distinct effect upon the trypanosomes, while IV is practically non-trypanocidal.

III

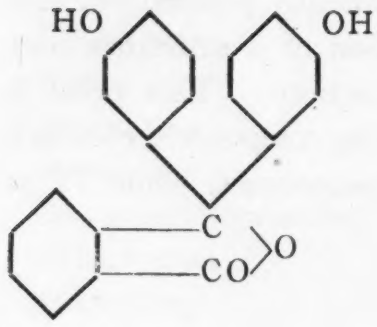
IV

Our work upon the aniline colouring matters was undertaken with the intention of starting with an indifferent organic compound, that is, one which has no effect upon the trypanosomes, and of combining with it various *radicles* in the hope of finding one which would have definite trypanocidal properties.

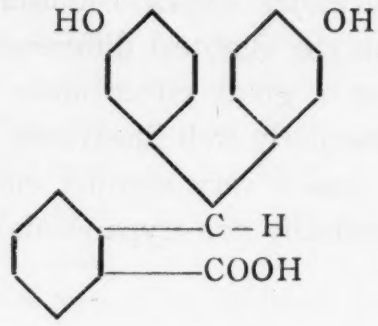
Our work has led us to the conclusion that the NH_2 group is such an active trypanocidal radicle, and we are accustomed to call it the 'trypanophobe' group.

For our starting point we took 'Phenolphthaleine,' V, which on being injected into the alkaline tissues, changes into, VI, a Triphenylmethane. This compound is of the same character as Malachite-Green and contains no NH_2 groups; we found it to be without effect upon the trypanosomes. We then proceeded to 'Fluorescein,' VII, and to different halogenic fluorescein derivatives, such as Eosin, Floxin, &c.; here, too, there was no trypanocidal effect. But when 'Rhodamine,' VIII, was used, it was found to have a very distinct effect upon the parasites; it possesses NH_2 groups.

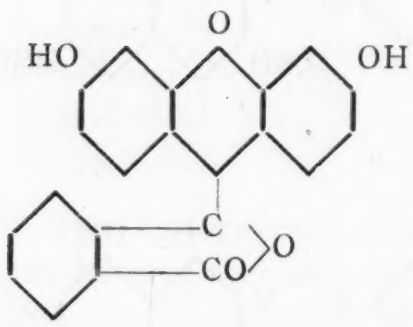
V



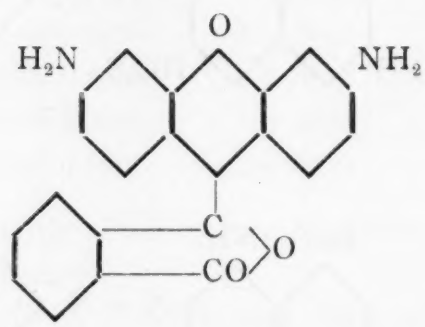
VI



VII



VIII



V. THE THERAPEUTIC VALUES OF ATOXYL AND ACETYLATED ATOXYL COMPARED (8)

Under this heading experiments were made with dogs, guinea pigs, and mice, and the effects of Atoxyl, of acetylated Atoxyl, and of acetylated Atoxyl followed by Mercury bichloride were compared. *Trypanosoma brucei* was the infecting parasite in each instance.

A. Dogs.

1. *Treatment by Atoxyl alone.*

Experiment 415.—Trypanosomes appeared in the peripheral circulation on the fourth day after inoculation; 5 c.cm. of a two per cent. solution of Atoxyl was given on the fifth day. The trypanosomes disappeared from the blood, but death ensued on the seventh day.

Experiment 421.—Trypanosomes appeared in the peripheral circulation on the fourth day after inoculation. A dose of 2 c.cm. of a five per cent. solution of Atoxyl was given on the fourth day and again on the fifth day. Death followed on the eighth day although the parasites had disappeared and remained absent.

Experiment 431.—Trypanosomes appeared in the peripheral blood on the fourth day. On the fourth and again on the fifth day a dose of 3 c.cm. of a five per cent. solution of Atoxyl was given. The parasites disappeared but reappeared

on the twelfth day; a third dose of the same solution of Atoxyl was then given, but death followed on the fourteenth day of the experiment although the parasites were not seen.

EXPERIMENT 436.—Trypanosomes appeared in the blood on the fourth day. On the fifth and again on the sixth day a dose of 3 c.cm. of a five per cent. solution of Atoxyl was given. The trypanosomes disappeared and remained absent, but death followed on the tenth day of the experiment.

EXPERIMENT 437.—Trypanosomes appeared in the peripheral circulation on the fourth day; a dose of 3 c.cm of a 5 per cent. solution of Atoxyl was given on the fifth day and on the sixth. The parasites disappeared and remained absent but the animal died on the ninth day.

From these five experiments it may be concluded that dogs infected with the strain of *Trypanosoma brucei* employed would die, though treated by Atoxyl, in about nine days.

2. *Treatment by acetylated Atoxyl alone.*

EXPERIMENT 416.—Trypanosomes appeared in the peripheral circulation on the fourth day. On the fifth day 5 c.cm. of a two per cent. solution of acetylated Atoxyl was given. The trypanosomes did not disappear so this dose was repeated on the seventh day; it was again repeated on the twelfth day, although the parasites had disappeared after the second dose. They reappeared on the thirtieth day; two doses, each of 5 c.cm. of a three per cent. solution were therefore given on the thirtieth and thirty-first days. The parasites disappeared after the last of these doses and did not reappear until the forty-fourth day, when two doses, each of 5 c.cm. of a three per cent. solution, were given on successive days and the parasites disappeared, to reappear once more on the fifty-sixth day. In spite of another dose then given of 5 c.cm. of a three per cent. solution, the animal died on the following day. Rats subinoculated on the fifteenth and thirty-seventh days remained negative.

A rat subinoculated on the fifty-first day, five days before death, became *infected*, however, after a prolonged incubation period of eleven days and died four days later.

EXPERIMENT 419.—Trypanosomes appeared in the peripheral circulation on the fourth day. On the fifth and again on the sixth day a dose of 5 c.cm. of a two per cent. solution of acetylated Atoxyl was given. The parasites disappeared but recurred on the eleventh day when two more doses, each of 5 c.cm. of the same solution were administered; although the parasites again disappeared, the dog died on the eighteenth day after inoculation.

Two dogs treated by acetylated Atoxyl alone lived eighteen and fifty-seven days.

3. *Treatment by acetylated Atoxyl followed by bichloride of Mercury.*

EXPERIMENT 334.—Trypanosomes appeared in the blood on the second day after inoculation. On the third, fourth and fifth days three doses of 5 c.cm. of a ten per cent. solution of acetylated Atoxyl were given; on the sixth and seventh days two doses of 10 c.cm. of the same solution were administered. On the eighth, the ninth and the tenth days 2 c.cm. of a one per cent. solution of Mercury bichloride were injected, and on the eleventh day were followed by a dose of

5 c.cm. of a ten per cent. solution of acetylated Atoxyl. The parasites disappeared from the blood on the fifth day of the experiment and were absent until the death of the animal on the forty-seventh day.

A rat was inoculated on the seventh and another on the twenty-fourth day of the experiment; neither of them ever became infected.

EXPERIMENT 420.—Trypanosomes appeared in the peripheral blood on the third day after inoculation. On the fourth and again on the fifth day 2 c.cm. of a five per cent. solution of acetylated Atoxyl was given. The parasites disappeared, and although they were still absent on the ninth and three succeeding days, 13 c.cm. of the same solution was given in four doses of increasing size; no toxic symptoms were observed; and on the fourteenth, and again on the fifteenth day 2 c.cm. of a 0·5 per cent. solution of Mercury bi-chloride was given. The parasites still remained absent, when on the twenty-ninth and thirtieth days two more doses of 5 c.cm. of the same solution of acetylated Atoxyl were injected and were followed on the two next days by two doses of 3 c.cm. of the same solution of Sublimate. The parasites remained absent until the death of the animal on the one hundred and fifth day; death was due in a large measure to very extensive mange.

On the twentieth, forty-first, and sixty-seventh days of the experiment respectively, two rats and one puppy were inoculated with large quantities of blood; none of them ever became infected.

EXPERIMENT 432.—Trypanosomes appeared in the blood on the second day. On the third and again on the fourth day 3 c.cm. of a five per cent. solution of acetylated Atoxyl was given; a dose of 5 c.cm. of a ten per cent. solution of acetylated Atoxyl was injected on the tenth and again on the eleventh day. This dose was repeated on the seventeenth and eighteenth days and was then followed on the nineteenth and twentieth days by two doses, each of 2 c.cm. of a 0·5 per cent. solution of Mercury bichloride. The parasites disappeared from the blood on the fourth day of the experiment and never reappeared. The animal died on the thirty-seventh day.

A rat was subinoculated from this animal on the sixteenth day; a rat and two mice on the twenty-eighth day, and a rat on the thirty-fifth day; none of these animals ever became infected.

Three dogs treated by acetylated Atoxyl lived from thirty-seven to one hundred and five days.

B. GUINEA PIGS.

1. *Treatment by Atoxyl alone.*

EXPERIMENT 406a.—Trypanosomes appeared in the blood on the fourth day. On the fifth day 1 c.cm. of a five per cent. solution of Atoxyl was given and the parasites disappeared, but death ensued on the seventh day.

EXPERIMENT 406b.—Trypanosomes appeared in the blood on the sixth day. On the seventh and again on the eighth day 1 c.cm. of a five per cent. solution of Atoxyl was given. The parasites disappeared, but the animal died on the ninth day.

EXPERIMENT 406c.—Trypanosomes appeared in the blood on the eighth day. On each of the two following days 1 c.cm. of a five per cent. solution of Atoxyl was given, and the parasites disappeared from the blood, but death followed on the fifteenth day.

Three animals treated by Atoxyl alone died in from seven to fifteen days.

2. *Treatment by acetylated Atoxyl followed by bichloride of Mercury.*

EXPERIMENT 407b.—Trypanosomes appeared in the blood on the sixth day. On the twelfth, thirteenth, twenty-second and twenty-third days, doses of 1·5 c.cm. of five per cent. solution of acetylated Atoxyl were given. These were followed on the twenty-fourth, twenty-fifth and twenty-sixth days by 0·5 c.cm. of a two per cent. solution of bichloride of Mercury. The animal died on the twenty-seventh day. No trypanosomes had been seen in the blood since the first dose of Atoxyl.

EXPERIMENT 407c.—Trypanosomes appeared in the blood on the eighth day. On the twelfth and thirteenth days 2 c.cm. of a five per cent. solution of acetylated Atoxyl were given and were immediately followed on the two succeeding days by doses of 1 c.cm. of a two per cent. solution of Mercury bichloride. The parasites disappeared after the first dose of Atoxyl and did not reappear, but the animal died on the sixteenth day

EXPERIMENT 414a.—Trypanosomes appeared in the blood on the eighth day. On the tenth, eleventh, and twelfth days, doses of 1·5 c.cm. of a five per cent. solution of acetylated Atoxyl were given. These were followed on the thirteenth, fourteenth, fifteenth, and sixteenth days by doses of 0·5 c.cm. of a two per cent. solution of Mercury bichloride, and on the seventeenth and eighteenth days by 3·5 c.cm. of the same solution of acetylated Atoxyl in two doses, and, finally, on the nineteenth and twentieth days, by two doses of 1 c.cm. of the same solution of Mercury bichloride. The trypanosomes disappeared from the blood on the second day of treatment and were still absent at the death of the animal from pneumonia on the sixty-seventh day.

Rats were subinoculated on the fourteenth, fortieth, and fifty-fourth days of the experiment; none of them ever became infected.

EXPERIMENT 414.—Trypanosomes appeared on the third day. Treatment was commenced on the eleventh day by five consecutive daily doses of 1·5 c.cm. of a five per cent. solution of acetylated Atoxyl. These were followed on the seventeenth and eighteenth days by two doses of 0·5 c.cm. of a two per cent. solution of bichloride of Mercury. The parasites disappeared from the blood on the second day of treatment and remained absent, but the animal died on the nineteenth day.

EXPERIMENT 419.—Trypanosomes appeared in the blood on the eighth day. Treatment was commenced on the thirteenth day with three consecutive doses of 1·5 c.cm. of a five per cent. solution of acetylated Atoxyl. It was continued on the twentieth and twenty-first days by two doses of 2 c.cm. of the same solution of Atoxyl, followed on the three next days by daily doses of 0·5 c.cm. of a two per cent. solution of Mercury bichloride, and on the twenty-sixth day by one more dose of 2 c.cm. of Atoxyl. The parasites which had disappeared from the blood on the second day of treatment remained absent until the forty-fifth day. On their reappearance their treatment was resumed by four consecutive daily doses of 2 c.cm. of the same solution of Atoxyl; these were followed on the fiftieth day by 1 c.cm. of a two per cent. solution of Mercury bichloride. The animal died on the next day, trypanosomes being absent from its blood.

Five guinea pigs treated by acetylated Atoxyl and bichloride of Mercury died in from sixteen to sixty-seven days (average about thirty days).

C. MICE.

Only one experiment was made, with the object of comparing the relative therapeutic values of Atoxyl and acetylated Atoxyl.

EXPERIMENT 443.—Two lots of three mice each were inoculated with approximately equal amounts of infected blood from a common source. All became infected on the third and fourth days. Each animal on the day it was found to be infected was treated; three mice received Atoxyl, and three acetylated Atoxyl (approximately equal quantities of the drug were given). Those treated with Atoxyl died on the fourth and fifth days; those with acetylated Atoxyl on the ninth and eleventh days.

From these experiments, so far as they permit comparisons and conclusions, we deduce for the animals concerned, that:—

Treatment by acetylated Atoxyl followed by Mercury is more efficacious than is treatment by acetylated Atoxyl alone; acetylated Atoxyl is of more value than Atoxyl; but that none of these methods is of practical value since death invariably occurred.*

VI. TREATMENT BY ATOXYL FOLLOWED BY ANOTHER DRUG

As explained in our former paper, it was thought that substances, ordinarily without therapeutic value, might be trypanocidal when administered after Atoxyl; it was this line of work which led to the discovery of the efficacy of Atoxyl combined with bichloride of Mercury in the treatment of trypanosome-infected rats.

The following combinations were tried and found to be valueless; the parasites generally reappeared in the blood in from three to four weeks after the cessation of treatment, and death followed in due course.

Atoxyl and Silver Nitrate.
Atoxyl and Lead Acetate.
Atoxyl and Quinine-cacodylate.
Atoxyl and Potassium Bichromate.
Atoxyl and Quinine.

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* It is true that trypanosomes could not be detected, either by subinoculation or direct examination, in some of these animals for many days before their death, and it must be asked whether they really died of trypanosomiasis or from some other cause, intoxication from the treatment for example; gross signs of overdosage were not observed. The same question must be asked concerning the deaths of many of the animals experimented with; this is particularly the case with the donkeys. Experiments 416 and 419 show definitely how infection may declare itself after being long dormant in thoroughly treated animals.

VII. TREATMENT OF ATOXYL FOLLOWED BY BICHLORIDE OF MERCURY

Under this heading are reported our attempts to apply the combined use of Atoxyl and bichloride of Mercury, found successful in the treatment of rats, to the cure of large animals infected with trypanosomes. Rabbits and donkeys were used in this series of experiments.

A. RABBITS.

1. *Controls, treated by Atoxyl alone.*

Three control rabbits which only received the same amount of Atoxyl as was used in the combined treatment by Atoxyl and bichloride of Mercury, all died in about fifty-five days after inoculation. In one rabbit (Experiment 302) death also occurred, although the dosage by Atoxyl was repeated continuously. In all, the infecting trypanosome was *Trypanosoma brucei.*

EXPERIMENT 302.—Fourteen days after inoculation trypanosomes appeared in the blood. Treatment was commenced nine days later. On the first day 1 c.cm. of a five per cent. solution of Atoxyl was given; one week later another dose of 1 c.cm. was injected, and on the day following 2 c.cm. The dosage of 2 c.cm. was then repeated for ten times at intervals of from five to eight days; so that in all 23 c.cm. of Atoxyl solution were given in twelve doses in forty-two days to a rabbit weighing 1805 grammes at the commencement of the experiment. Trypanosomes reappeared in the peripheral blood in forty-three days after the commencement of treatment, and the animal died three days later weighing 1560 grammes.

Rabbits treated by Atoxyl alone, whether continuously or not, died in about fifty to sixty days after inoculation.

2. *Treatment by Atoxyl followed by bichloride of Mercury.*

(*a*) Rabbits infected with *Trypanosoma brucei*

EXPERIMENT 288.—After fifteen days trypanosomes appeared in the blood of a rabbit inoculated on January 7. Six days later treatment was commenced, the animal had already lost 110 grammes of an original weight of 2350 grammes. Three c.cm. of a five per cent. Atoxyl solution were given in four doses during a period of eleven days; 2 c.cm. being given in the last three days. Three days after the last dose of Atoxyl the dosage by bichloride of Mercury was commenced, and 4 c.cm. in four doses were given in five days' time. The blood was examined daily for one hundred and forty-seven days, until January 10th, when the animal died. No macroscopic cause of death was seen at the autopsy; there were no signs of trypanosomiasis. Mice were subinoculated from this animal one day after treatment was stopped and at fourteen, twenty-five, fifty-one and one hundred and forty-one (3 mice) days from that date. A young puppy was inoculated (with 10 c.cm. of blood) at the time of the autopsy. All these animals were carefully observed for a sufficient length of time; none of them were ever observed to become infected with trypanosomes. A rat inoculated on the third day after the first dose of Atoxyl became infected in nine days and died three days later.

EXPERIMENT 289.—Six days after its inoculation on January 24th, trypanosomes appeared in the peripheral blood of an adult female rabbit. Treatment was commenced five days later, and 3·5 c.cm. of five per cent. Atoxyl were given in four doses on four successive days. On the four following days 4 c.cm. of a two per cent. solution of Mercury bichloride was given in four equal doses. In spite of very careful examination trypanosomes could never be found in the peripheral blood of the animal, although it steadily lost weight; thus at the time of inoculation it weighed 2020 grammes, at the commencement of treatment 1850 grammes, and at seventy days after the cessation of treatment 1712 grammes. A puppy was subinoculated at this time but it never became infected. Because of this loss of weight a second course of treatment was commenced eighty-two days after the stoppage of the first course; 8 c.cm. of Atoxyl was given in three approximately equal doses on three successive days and was immediately followed on the two next days by two doses, each of 2 c.cm., of a two per cent. solution of bichloride of Mercury. The animal's weight soon commenced to increase, and in six weeks 160 grammes was gained. The animal, unfortunately, died from pneumonia on August 8th, two hundred and seventy days after inoculation. Its blood had been examined almost daily and trypanosomes were never seen, nor were they ever found in a rabbit and two mice subinoculated with large amounts of blood from it on July 12th and July 28th respectively.

EXPERIMENT 291.—An adult male rabbit was inoculated on January 24th, 1907. After ten days trypanosomes appeared in its blood. Four days later treatment was commenced and 4 c.cm. of five per cent. Atoxyl solution was given in equal doses during five days. This was immediately followed on four successive days by 4 c.cm., given in four equal doses, of a two per cent. solution of bichloride of Mercury. The animal's blood was examined carefully, but trypanosomes were never again seen, and the animal was still living on the 3rd March, 1908, four hundred and three days after inoculation.

Rats were subinoculated from this animal at fourteen, forty-five, seventy-five, one hundred and six, one hundred and thirty-six, and one hundred and sixty-seven days after the cessation of treatment. All of them have been carefully examined and none of them have ever been found to be infected.

EXPERIMENT 350.—Trypanosomes were first seen in the blood of an adult female rabbit on April 29th, 1907, twenty-five days after its inoculation. Treatment was commenced next day, on April 30th, and 10 c.cm. of Atoxyl solution was given in four doses during the next seven days; 6 c.cm. were given in the last two doses. This was immediately followed by 4 c.cm. of a two per cent. solution of bichloride of Mercury, given in four equal doses on four successive days. The animal was still alive on March 3rd, 1908, three hundred and thirty-three days after inoculation. Trypanosomes were never seen in its blood, nor did they ever appear in the blood of rats subinoculated from it on the last day of treatment, and at fifty-eight, eighty-nine, and one hundred and twenty days from that date.

EXPERIMENT 356.—Trypanosomes appeared in the peripheral blood of an adult female rabbit seven days after its inoculation. Six days later treatment was commenced, and 2 c.cm. of a ten per cent. solution of Atoxyl was given in one dose. This dose was repeated seventeen days later, and then was immediately followed by three equal doses of 1 c.cm of a two per cent. solution of Mercury bichloride, given on three successive days. Trypanosomes were not seen in the animal's blood from the date of the first injection of Atoxyl to its death, forty-six days later (no autopsy; cause of death unknown), nor were trypanosomes seen in the blood of a rat subinoculated from it at thirty-one days from the commencement of treatment.

Of five rabbits infected with *Trypanosoma brucei* and treated by

Atoxyl followed by bichloride of Mercury, one died in forty-six days and four were apparently cured; this combined treatment of rabbits infected with *Trypanosoma brucei* therefore is much superior to the treatment by Atoxyl alone, and will sometimes effect a cure.

(*b*) Rabbits infected with *Trypanosoma gambiense*

EXPERIMENT 323.—Trypanosomes appeared after forty-one days in the blood of a rabbit inoculated on February 26th. Treatment was commenced sixteen days later and 7 c.cm. of five per cent. Atoxyl solution was given, in four doses at equal intervals, during the next twelve days. This was immediately followed during the succeeding ten days by 4 c.cm. of bichloride solution, given in four equal doses at approximately equal intervals. The rabbit's blood was examined practically daily for one hundred and forty-one days, until October 5th, when it died (cause not known; no autopsy). Trypanosomes were never seen in it after the treatment was stopped, nor in mice subinoculated from it thirty days from that time

EXPERIMENT 326.—After eighteen days trypanosomes were found in the ear blood of a rabbit inoculated on February 26th, 1907. The parasites were constantly present when treatment was commenced forty-three days later, and 8 c.cm. of five per cent. Atoxyl solution was given in four doses at approximately equal intervals during the next fifteen days. This was immediately followed by 4 c.cm. of Mercury bichloride solution, given in four doses at almost equal intervals during the succeeding ten days. The rabbit's blood was examined daily for forty-nine days and at intervals of two or three days until January 26th, 1908, eleven months after inoculation, when it was killed. Trypanosomes were never seen in it after treatment was stopped, nor in the three rats and two mice subinoculated from it just at the end of treatment and a month after treatment had stopped.

EXPERIMENT 327.—After thirty-one days trypanosomes were found in the peripheral blood of an adult male rabbit inoculated on February 22nd, 1907. Treatment was commenced thirty days later, and 8 c.cm. of five per cent. Atoxyl solution was given in four doses during the next twelve days. This was followed in the succeeding ten days by 4 c.cm. of one per cent. Mercury bichloride solution, given in four equal doses at almost equal intervals of time. The rabbit's blood was examined daily after the cessation of treatment for one hundred and eighty-four days and, after that, at intervals until February 3rd, 1908, over eleven months after inoculation, when the animal was killed because of severe skin disease. Trypanosomes were never seen in its blood after the treatment was stopped, nor in mice and rats subinoculated, respectively, a month after the stoppage of treatment and at the autopsy.

EXPERIMENT 329.—After twenty-six days trypanosomes appeared in the peripheral blood of a rabbit. Treatment was commenced thirty-two days later, and 11 c.cm. of five per cent. Atoxyl solution was given in five doses during the next twelve days; just over half of the drug was given in the last two days. This was followed during the next nine days by 7 c.cm. of bichloride solution, given in four doses; just half of this drug was given on the first two days. After cessation of the treatment, the rabbit's blood was examined daily for one hundred days, when it died (cause not known; no autopsy). Trypanosomes were never seen in its blood, nor did they appear in the blood of a mouse inoculated at the termination of the treatment nor in rats inoculated at respectively thirteen and seventy-four days from that time.

The disease produced in rabbits by *Trypanosoma gambiense* may run a very chronic course; it is therefore better to say nothing concerning Experiments 323 and 329. Experiments 326 and 327 may, however, be safely considered to have been cured of an infection by *Trypanosoma gambiense.*

B. DONKEYS.

The outcome of this series of experiments was extremely disappointing. It was commenced immediately after the favourable results had been obtained in the treatment of rats by means of combined Atoxyl and Mercury bichloride. There is no doubt that in the first of the succeeding experiments the disease was allowed to go too far before the commencement of treatment; but even when, as in the later experiments, treatment was commenced early, it was found impossible to save a single animal.

In all the donkey experiments, as a general rule, the temperature, taken twice daily, became higher when the trypanosomes reappeared in the peripheral blood; whether trypanosomes were present or not it was often noticed that an elevated temperature fell immediately after the administration of another dose of the drug, and it was frequently observed that animals inoculated at such a time, that is, when the examination of the blood was negative and the temperature was high, often became infected. As a rule, therefore, a high temperature was accepted as a definite indication for a dose of Atoxyl.

1. *Controls, treated by Atoxyl alone.*

The disease was allowed to run its course, without treatment, in two donkeys; trypanosomes appeared in the blood on the third day, and they died after a typical illness, with remitting and intermitting fever going up to 104° or 105° F., in eighteen and twenty-four days respectively; at the post-mortem typical signs of trypanosomiasis were present.

Treatment was commenced on the twentieth day in a third animal, inoculated at the same time, when it was already practically moribund; it died on the twenty-third day.

EXPERIMENT 9.—Trypanosomes appeared four days after inoculation. They increased in numbers slowly and treatment was not commenced until the twenty-sixth day, when 10 c.cm of a two per cent. solution of Atoxyl was given. This dose was repeated on the thirty-second and thirty-third days because of a recurrence

of the trypanosomes, which had disappeared after the first inoculation. The parasites then again left the blood, only to reappear on the forty-first day. Death followed on the forty-sixth day.

EXPERIMENT 391.—Trypanosomes appeared in the blood on the fifth day after inoculation. On the thirty-first day treatment was commenced by a dose of 10 c.cm. of a ten per cent. solution of Atoxyl. This was repeated, as the parasites reappeared in the blood, on the thirty-second, thirty-third, thirty-eighth, forty-second, and forty-fifth days. Since the last dose of Atoxyl did not drive out the trypanosomes, a dose of 20 c.cm. of a twenty per cent. solution of Atoxyl was given. The parasites disappeared then from the blood, but the animal died three days later on the forty-eighth day.

EXPERIMENT 393.—Trypanosomes appeared in the blood on the fourth day. Treatment was commenced on the thirty-fifth day by giving four consecutive daily doses of 10 c.cm. of a ten per cent. solution of Atoxyl. The parasites disappeared from the blood, only to reappear on the forty-sixth day, when 10 c.cm. of a twenty per cent. solution of Atoxyl was given. The parasites once more disappeared to return again on the fifty-eighth day, when a dose of 20 c.cm. of a twenty per cent. solution of Atoxyl was given. The parasites again disappeared from the blood and did not return before death on the sixty-seventh day. Three rats were subinoculated from this animal on the thirty-seventh, sixty-first and sixty-sixth days, when trypanosomes were not present. All three of these animals were carefully examined for periods of from three to four months; none of them ever became infected.

EXPERIMENT 2.—Trypanosomes appeared in the blood on the fourth day. Treatment was commenced on the thirty-fourth day by 10 c.cm. of a ten per cent. solution of Atoxyl. This dose was repeated daily on the three following days and again, when twice that amount was given, on the forty-fourth day. But the trypanosomes, which had been absent since the second dose, reappeared on the forty-seventh day and the animal, in spite of a dose of 10 c.cm. of a ten per cent. solution of 'Afridol,' died on the day following. Rats subinoculated on the thirty-sixth and forty-sixth days never became infected.

EXPERIMENT 393.—Trypanosomes appeared in the blood on the fourth day. Treatment was commenced on the twenty-sixth and two following days by giving daily 10 c.cm. of a ten per cent. solution of Atoxyl. In spite of the most careful nursing, the animal died on the thirty-seventh day, although the trypanosomes did not reappear in the peripheral blood. Two rats subinoculated on the thirty-first day of the experiment never became infected.

EXPERIMENT 17.—Trypanosomes appeared in the blood on the third day. Treatment consisted of 10 c.cm. of a ten per cent. solution of Atoxyl, given on the twenty-second, twenty-seventh and twenty-eighth days and, thereafter, once a week until the death of the animal on the ninety-seventh day. The trypanosomes disappeared from the blood immediately after the first dose of Atoxyl, and did not reappear until the seventy-eighth and seventy-ninth days, when they again disappeared and were absent at death. The chart of this animal shows a typical high fever until the commencement of treatment; then the temperature remained approximately normal with slight rises until the seventy-sixth day, when it again became high and continued to run an elevated course.

EXPERIMENT 19.—Trypanosomes appeared in the peripheral blood on the third day. Treatment was commenced on the twenty-fourth day, when 10 c.cm. of a ten per cent. solution of Atoxyl was given. This dose was repeated on every second day from that time until the death of the animal on the seventy-third day. Trypanosomes were not seen in the blood after the first dose of Atoxyl. Rats inoculated on the twenty-fifth and thirty-eighth days of the experiment, however, *became infected* after a prolonged incubation of about two weeks and died in due

course. The temperature of this donkey, although irregularly febrile, did not run as high as was usually the case; it did not go above 103° after treatment was commenced.

EXPERIMENT 14.—Trypanosomes appeared in the blood on the fifth day. Treatment was commenced on the seventeenth day by injection of 10 c.cm. of a ten per cent. solution of Atoxyl. This same dose was repeated daily—Sundays excepted—from then until the ninety-fourth day of the experiment. The trypanosomes disappeared from the blood after the first dose of Atoxyl and did not reappear until the seventy-fourth and seventy-fifth days, when they once more disappeared until the ninety-fifth day. They disappeared once more and were not seen again before death on the one hundred and fourth day. Rats subinoculated just before the second dose of Atoxyl never became infected, while rats inoculated on the fifty-fourth and sixty-eighth days both became *infected* after incubation periods slightly longer than usual and died in due course.

From these experiments it appears that daily doses of 10 c.cm. of a ten per cent. solution of Atoxyl, and occasionally twice that amount, could be given to an adult donkey without exciting acute signs of poisoning; but even these large doses were insufficient to keep the trypanosomes out of the blood and the animals invariably died, whether the parasites were to be found in the blood or no, in from twenty-three to one hundred days, usually in about fifty days.

2. *Treatment by Atoxyl and bichloride of Mercury.*

(*a*) Symptomatic treatment

In the following series of experiments treatment was given practically only when it seemed necessary because of the presence of trypanosomes, or of a high temperature.

At first weaker solutions of Atoxyl were used; as it became apparent that they were inefficacious they were strengthened, until the saturated twenty per cent. solution was currently employed; this same strength of the solution was used in all the later experiments.

EXPERIMENT 11.—Trypanosomes appeared in the blood five days after inoculation. On the eighth and ninth days treatment was commenced by the daily injection of 10 c.cm. of a five per cent. solution of Atoxyl; on the twelfth and thirteenth days, doses of 1 c.cm. of a one per cent. solution of Sublimate were given; on the fourteenth day four times that amount of the drug was administered. On the fifteenth, eighteenth, twenty-ninth, thirty-fourth and thirty-fifth days 10 c.cm. of Atoxyl was injected. The trypanosomes disappeared from the blood on the second day of treatment; there were one or two considerable elevations in temperature but the parasites remained absent from that time until the twenty-ninth day, when 10 c.cm. of a ten per cent. solution of Atoxyl was given for three days, and was immediately followed by 10 c.cm. of a two per cent. solution of bichloride of Mercury. The parasites disappeared from the blood as a result of this treatment, but reappeared on the forty-sixth day. Three daily doses of 10 c.cm.

of a ten per cent. solution of Atoxyl were then given and the parasites once more disappeared, only to reappear later and remain more or less constantly present until the death of the animal on the eighty-third day.

EXPERIMENTS 8, 10 and 12.—Only the first of these experiments is given in full. The treatment was practically identical in all. The course of the disease was much the same and the same disappointing result ensued in each.

EXPERIMENT 8.—See Chart I.

EXPERIMENT 10.—Trypanosomes appeared in the blood on the fourth day. Treatment was commenced on the eleventh day. The parasites reappeared in the blood after a prolonged absence on the thirty-first day and again at intervals until the death of the animal at one hundred and seventy-five days.

It is interesting to note that a rat subinoculated two days before the recurrence of the trypanosomes in the early part of the infection remained negative, while another rat inoculated during a very slight rise in temperature (102°) at a time when no trypanosomes were present in the blood for ten days before or after, became *infected*.

EXPERIMENT 12.—Parasites appeared in the blood on the fourth day. Treatment was commenced on the eleventh day. The parasites were present in the blood at long intervals until the death of the animal at two hundred and three days.

Subinoculations were made from this animal on the twenty-ninth, forty-fifth, ninety-first, and one hundred and first days. That made on the forty-fifth day was negative, although trypanosomes had been present in the blood only four days previously; those made on the twenty-ninth and one hundred and first days were also negative, while that made on the ninety-first day, although trypanosomes were not seen in the blood for approximately a month before or after, was *positive*. The temperature was not elevated at this time.

EXPERIMENT 18.—Trypanosomes appeared in the blood after three days. Treatment was commenced on the eighth day by the injection of 10 c.cm. of a twenty per cent. solution of Atoxyl. This dose was repeated on the following day and on the seventeenth and eighteenth days, while one dose of 5 c.cm. of a five per cent. solution of bichloride of Mercury was given on the tenth day. The trypanosomes only left the blood for two days after the first dose of Atoxyl, they then reappeared. In spite of the repeated doses of Atoxyl they persisted, and the animal died on the nineteenth day.

The early persistence of the parasites, after vigorous treatment, in this experiment is extraordinary; they seem to have suddenly acquired Atoxyl-resistant characters. Such an occurrence is unique in our experience.

EXPERIMENTS 22-26.—In this series of experiments the dose of Atoxyl was always 10 c.cm. of a twenty per cent. solution, and the dose of bichloride 10 c.cm. of a two per cent. solution. As a rule a series of two doses of Atoxyl followed by one of bichloride of Mercury was given weekly on three successive days. If trypanosomes appeared in the blood or if the temperature were high, this dose was repeated at closer intervals, while if the temperature were normal and the parasites absent it was occasionally omitted for one week.

EXPERIMENT 25.—This experiment is given in full (Chart II.). It is a typical example of the course and fatal issue of the disease in the remaining experiments.

EXPERIMENT 22.—The parasites appeared in the blood in two days. Treatment was commenced at once. The temperature ran an irregular course and the parasites appeared at irregular intervals, until death ensued on the seventy-ninth day.

EXPERIMENT 23.—Trypanosomes appeared in the blood on the second day. Treatment was commenced on the seventeenth day. The parasites, which recurred early in the treatment, were absent for six weeks toward its end, but they eventually reappeared and the animal died on the one hundred and twenty-second day.

A rat subinoculated on the twenty-eighth day became *infected* after an inoculation period of 23 days, although trypanosomes had not been seen in the blood for a week and the treatment of three doses of Atoxyl and two of Mercury bichloride had only ended five days previously. The temperature was not high (99·2°), and trypanosomes did not appear in the blood for two weeks later.

EXPERIMENT 24.—Parasites appeared in the blood on the third day. Treatment was commenced four days later. The parasites were very persistent at the first part of this experiment, although they were absent during the last three weeks. The animal died on the thirty-third day.

Two rats subinoculated while the parasites were absent, on the day of death and ten days previously, both became *infected*.

EXPERIMENT 26.—Parasites appeared on the twenty-second day. Treatment commenced three days later. Death followed on the forty-second day.

(*b*) Routine treatment

Since symptomatic treatment failed, the continuous, routine administration of large doses of both drugs was adopted as a last resort; it, too, failed.

EXPERIMENTS 16 and 21.—The treatment after the commencement was the same in both these animals. A dose of 10 c.cm. of a twenty per cent. solution of Atoxyl was given on the first and second days, and was followed by 10 c.cm. of a two per cent. solution of bichloride of Mercury on the third day. This treatment was repeated twice weekly; on Sundays no treatment was given.

EXPERIMENT 16.—Trypanosomes appeared in the blood on the fourth day. Treatment was commenced on the twenty-fourth by three successive doses of 10 c.cm. of a ten per cent. solution of Atoxyl. Treatment as described above was thenceforth continued until the one hundred and sixth day, when the parasites, which had been continually absent since the second day of treatment, reappeared. Trypanroth in daily doses of 10 c.cm. of a one per cent. solution was then given on six consecutive days. The parasites failed to disappear and the animal died on the one hundred and twentieth day.

Animals subinoculated on the fiftieth day did not become infected, while one inoculated on the ninety-third day, thirteen days before the reappearance of the parasites, became *infected* after an incubation of seven days.

EXPERIMENT 21.—Trypanosomes appeared in the blood on the third day. Treatment was commenced on the twenty-sixth day, and in the next fourteen days seven doses of 10 c.cm. of a ten per cent. solution of Atoxyl were given. The parasites, which disappeared after the first two doses of Atoxyl, reappeared twice during this course of medication. The Atoxyl was followed by 16 c.cm. of a two per cent. solution of Sublimate, given in three doses, on two consecutive days. From the forty-sixth to the one hundred and fifth day the treatment indicated above was given regularly. The parasites disappeared at once and remained absent to examination and subinoculation until the ninetieth day, when they once more reappeared and were constantly present until death on the one hundred and twentieth day.

It is interesting to note that a rat subinoculated with blood taken during the first course of treatment with Atoxyl, although trypanosomes were present, only became *infected* after an incubation of sixteen days.

EXPERIMENT 20.—Trypanosomes appeared on the fourth day. On the sixth, treatment was commenced and was continued until two days before death on the thirty-eighth day. For two days 10 c.cm. of a twenty per cent. solution of Atoxyl was given daily and on the third day 10 c.cm. of a two per cent. solution of bichloride of Mercury was administered. This treatment was repeated twice weekly. The trypanosomes disappeared from the blood five days after treatment was commenced and never reappeared.

A rat subinoculated on the thirty-fourth day, four days before death, became *infected* after a long incubation of twenty-three days. Another rat subinoculated on the seventeenth day never became infected.

Donkeys treated by Atoxyl followed by bichloride of Mercury all died in from about thirty-three to two hundred days (average one hundred days), in spite of large doses.

VIII. TREATMENT BY TRYPANROTH FOLLOWED BY BICHLORIDE OF MERCURY

This series of experiments was undertaken to ascertain whether Sublimate would have the same value when given after another trypanocidal substance as it possesses when following Atoxyl. There was a slight difficulty in carrying out these experiments, since Trypanroth was not always able to prevent an early death from the disease.

EXPERIMENT 800.—Trypanosomes appeared in the blood of six rats on the first day after inoculation. A dose of 1 c.cm. of a one per cent. solution of Trypanroth was at once given and was repeated daily on the ten following days; the parasites had disappeared from each of the rats on the sixth day of the experiment. Three of the rats then received on the three following days doses of 0·5 c.cm. of a one per cent. solution of bichloride of Mercury; three received no further treatment. The parasites reappeared in all six rats and death followed in from two to four days. Those which received only Trypanroth died in sixteen, seventeen, and eighteen days; those which received Trypanroth and Sublimate in seventeen, nineteen, and twenty-two days.

EXPERIMENT 802.—Trypanosomes appeared in six rats on the third day after inoculation. One c.cm. of a one per cent. solution of Trypanroth was at once given and was repeated on the six following days. The parasites disappeared from the blood on the eighth day of the experiment in five of the rats; one died. Three doses, on consecutive days, of 1·5 c.cm. of a one per cent. solution of Mercury bichloride were then given to three of the rats; the remaining two had no further treatment. The parasites ultimately reappeared in all of them and death followed as usual in from two to four days. Those which received only Trypanroth died in fifteen and sixteen days from the commencement of the experiment; those which received the combined treatment in fourteen, twenty-five, and forty-eight days.

From these experiments it seems that combined treatment by Trypanroth, followed by bichloride of Mercury, is superior to treatment by Trypanroth alone, but it is far inferior to the treatment by Atoxyl and Mercury.

IX. SUMMARY AND CONCLUSIONS

I. Certain definite rules which must be followed in the experimental therapeutics of trypanosomiasis are insisted upon.

II. The after history of the rats dealt with in our first papers ([1, 2]) is referred to. The Atoxyl-treated controls all eventually died of trypanosomiasis; those treated by Atoxyl followed by bichloride of Mercury, and by Atoxyl followed by Donovan's solution, which were thought to be cured, have never had recurrences, but they were not immune to re-inoculation.

III and VI. Several drugs and combinations of drugs found to be inefficacious in the treatment of infected rats are named.

IV. None of the colouring matters employed were of much value; it is suggested that the active radicle in *trypanocidal aniline derivatives* is the 'trypanophobe' group, NH_2.

V. In the treatment of dogs, guinea pigs, and mice, the comparative value of (1) acetylated Atoxyl followed by bichloride of Mercury, (2) of acetylated Atoxyl, and (3) of Atoxyl, is as the order in which they are named; none of these methods is usually able to definitely cure well-established infections in these animals.

VII. A. Atoxyl followed by bichloride of Mercury is found to be much superior to Atoxyl alone in the treatment of rabbits infected with *Trypanosoma brucei*; the latter is also effective in the treatment of rabbits infected with *Trypanosoma gambiense.*

B. Atoxyl and Mercury combined are distinctly superior to Atoxyl alone in the treatment of donkeys infected with *Trypanosoma brucei,* but neither method is able to cure a well-established infection.

VIII. Treatment of rats infected with *Trypanosoma brucei* by Trypanroth followed by bichloride of Mercury is superior to treatment by Trypanroth alone, but inferior to the combined treatment by Atoxyl and bichloride of Mercury.

IX. Many subinoculations were made during the work to ascertain whether the animals experimented with were infected. The great majority of subinoculations made from animals in whose blood trypanosomes could not be seen were negative; thirteen, made under apparently identical circumstances, were positive. An examination of these subinoculations is interesting.

Rats became infected although trypanosomes were not seen in the animal from which the blood was taken for over a month both before and after the subinoculation. They became infected even although large doses of Atoxyl or of Atoxyl and Mercury had been given to the infecting animal only a day previously. Blood taken during a rise in temperature was often infective; but blood taken while the temperature was no higher than usual, and at a time neither preceding nor following a rise, was also infective; blood taken during the customary *ante-mortem* fall of temperature was infective in four of these thirteen experiments, although trypanosomes were absent from it.

The chief interest in these successful subinoculations lies in the fact that the incubation period was greatly lengthened; while the course of the disease, once the parasites appeared in the blood, was normal, and the rats died about three days later.[11] In six of these thirteen subinoculations the incubation period was over fifteen days, in three over twenty, the longest was twenty-six days; only once, and then in blood taken from a dying animal, was the incubation so short as four days. Although other explanations suggest themselves, these observations seem to be in harmony with our belief that recurrences in apparently-cured, trypanosome-infected animals are due to the production and persistence of some resistant developmental form of the parasite, and not merely to the acquirement of 'chemotherapeutic resistant' properties by it.

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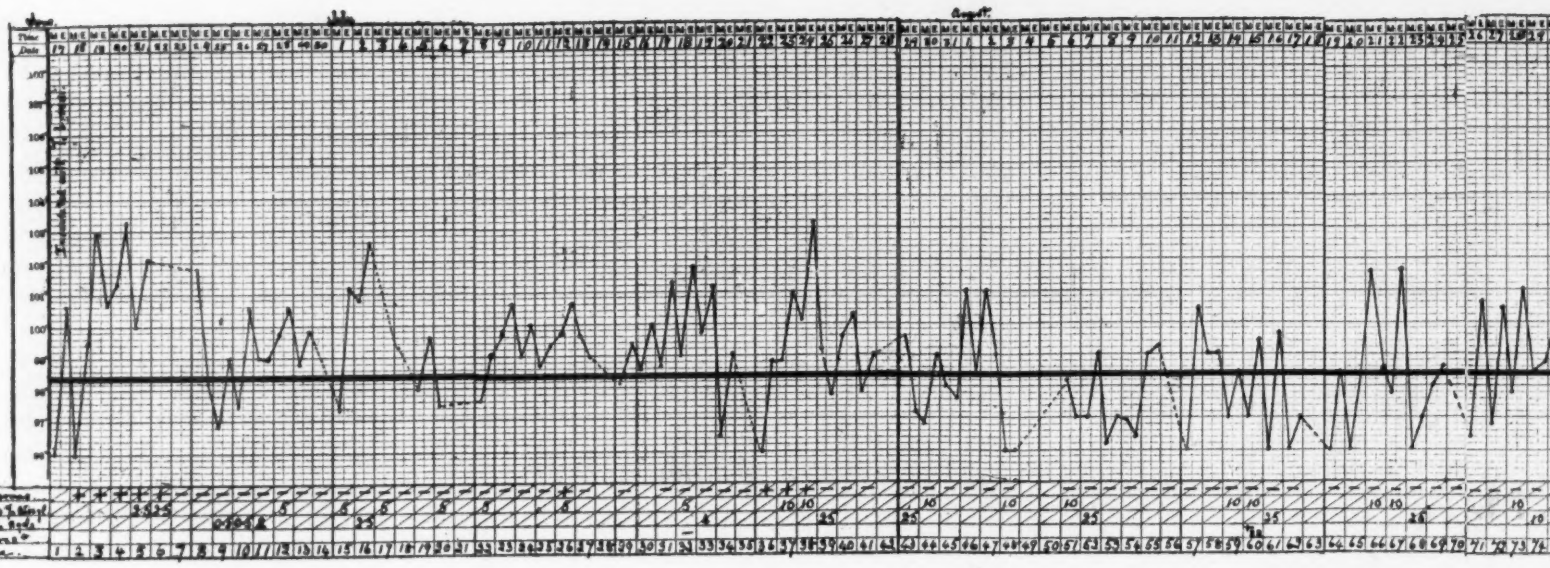
CHART I.

DONKEY No. 8.

Inoculated with *T. brucei.*

Treated by Atoxyl and bichloride of Mercury.

Trypanosomes
(if present = +
(if absent = −



* The incubation period of successful subinoculations is given in days.

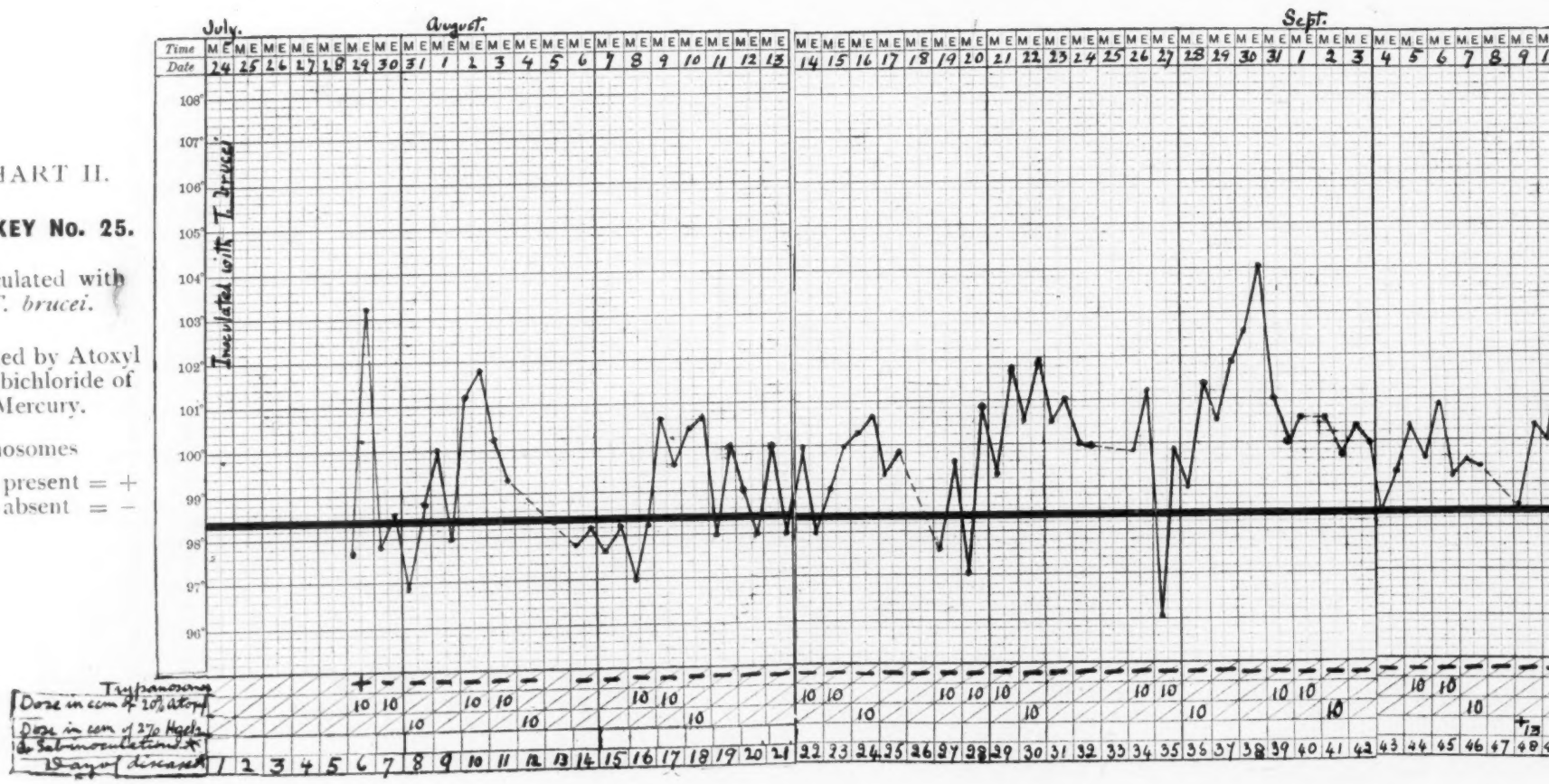
CHART II.

DONKEY No. 25.

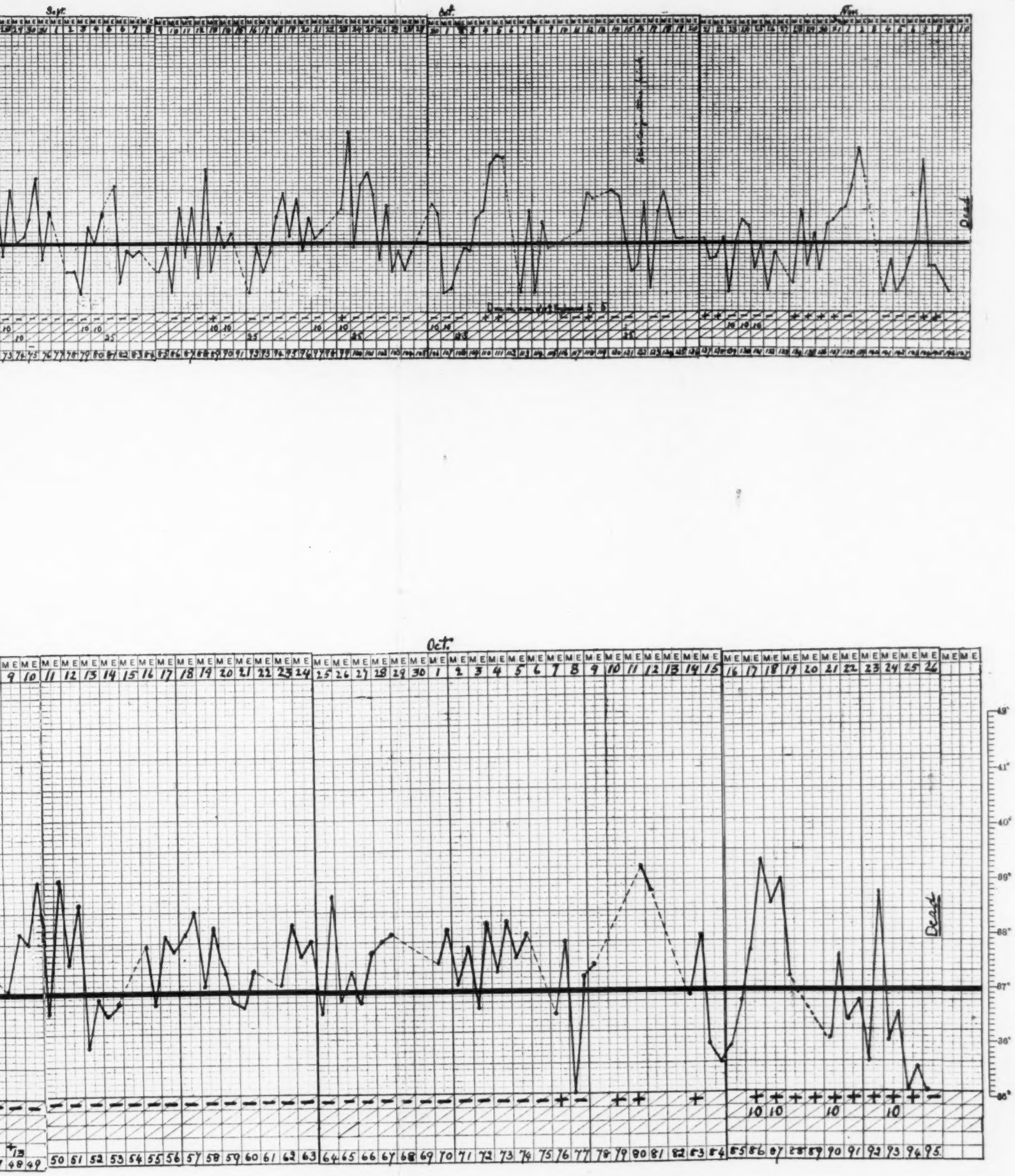
Inoculated with *T. brucei.*

Treated by Atoxyl and bichloride of Mercury.

Trypanosomes
(if present = +
(if absent = −



* The incubation period of successful subinoculations is given in days.


Sept.
Oct.
Nov.
Oct.
Dead
49°
41°
40°
39°
38°
37°
36°
35°

AN UNUSUAL CASE OF GOUNDOU

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(Received for publication 17 June, 1908)

Whilst at Kanre Lahun, on the Liberian frontier, I chanced to see a case of Goundou which presented some unusual and interesting characters.

The patient was a Mendi man, about 22 years of age. He stated that about five years ago the usual tumours began to grow, their appearance being preceded by a severe attack of yaws and constant severe headache. He said he had never had syphilis. About a year later, a third tumour began to develop on the left side of the face in the malar region. This third tumour is slowly increasing in size, whilst the others (nasal) have not increased to any appreciable extent during the past two years. It is only within the last few months that a discharge has been noticed from the right nostril, and no discharge has ever been observed from the left. Constant severe headache has been present, preventing him from working and causing loss of sleep. When he stoops down the pain is greatly increased.

On examination the patient is seen to be of poor physique.

FACE. On each side of the nose, springing from the nasal processes of the superior maxillae, a tumour is seen. They are symmetrical, sessile, and of bony hardness. Somewhat oval in shape, the long axis being downwards and outwards, they are smooth with normal skin freely moveable over them. They are dull on percussion. There is a foul discharge coming from the right nostril. The passage of tears through the nasal ducts is not interfered with. The tumours practically fill the nostrils, and the patient is unable to breathe through the nose. The sense of smell is totally lost; he is unable to distinguish the odours of tobacco, ammonia, and peppermint.

On the left side of the face a third tumour is seen in the malar region close to, but separate from, the previously described ones. In size and shape it is about that of half a hen's egg, but it is not entirely smooth and regular, as on its upper and outer aspect a protuberance can be felt. The long axis of the tumour is upwards and outwards, and the outer canthus of the eye is dragged with it. It

does not invade the orbital cavity. The tumour is of bony hardness, is not painful on being handled, is dull on percussion, and the skin over it is normal and freely moveable. Pain is frequently felt radiating to the tempero-maxillary articulation. There is no sign of oedema or 'egg-shell' crackling. This tumour has developed more rapidly than the usual tumours.

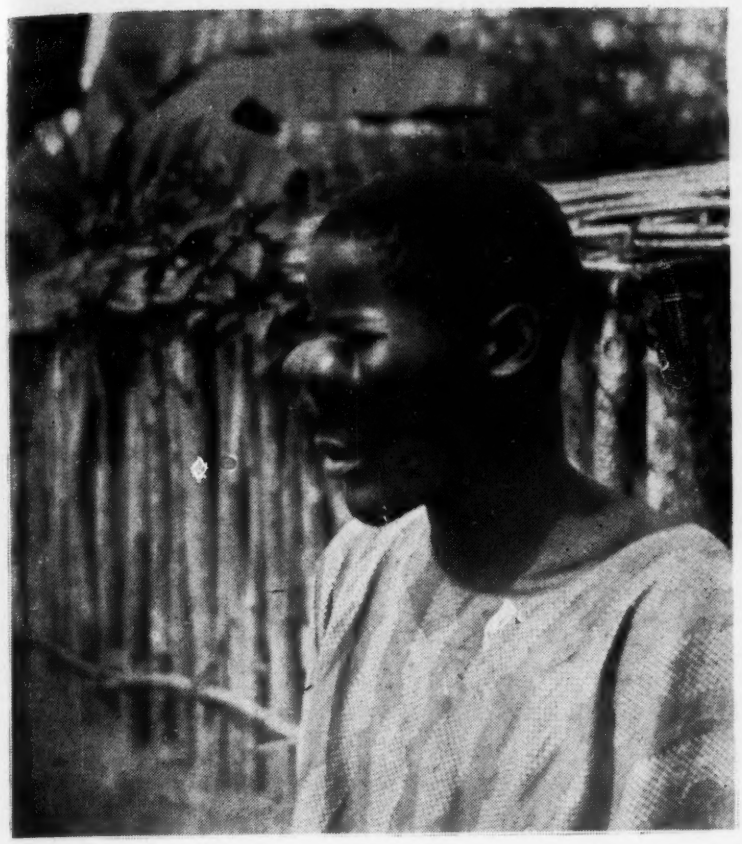
Examination of the mouth shows nothing abnormal, the patient possessing a perfect set of teeth with none missing. His palate is normal, though somewhat low, and both sides are symmetrical. His chest is flat; his breasts are unduly developed, and the nipples are large and prominent. His heart and lungs are normal. The abdominal organs are also normal. His legs are remarkable for the great forward curving of the tibiae, without any thickness of their anterior borders, which are quite 'sword-like' in sharpness. From time to time sharp shooting pain is experienced in the legs. The reflexes are normal. Scars of old yaws can be seen on his chest and arms.

Treatment. Potassium iodide in increasing doses, bromides, phenacetin, &c., were all apparently useless.

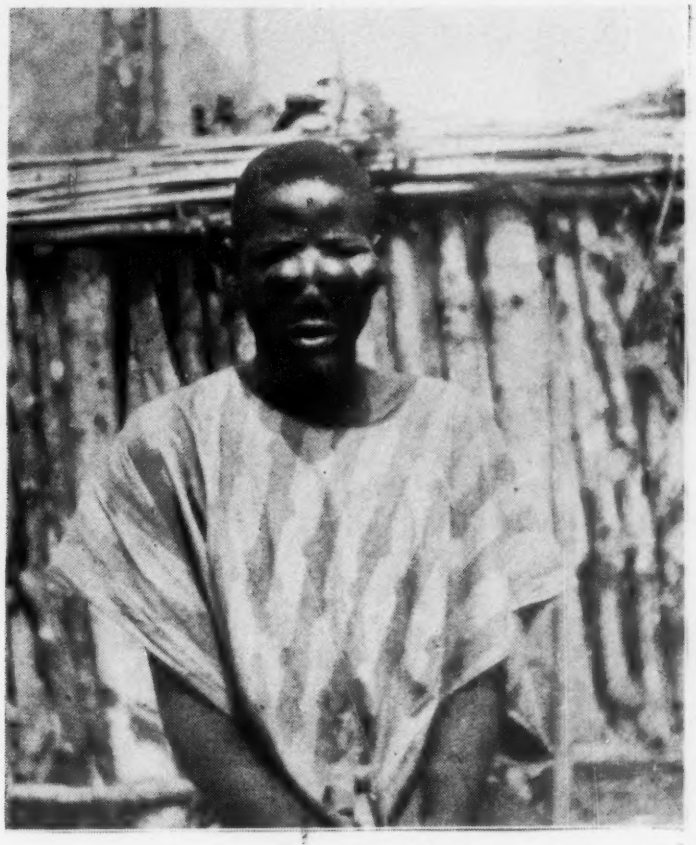
The chief reason for venturing to bring forward this case is the presence of the third tumour. I have been unable to find that such has been described before associated with goundou. The associated curvature of the tibiae has been noted already by others, but it is not referred to in most text books dealing with tropical disease, and the same observation largely holds in reference to the continued patency of the nasal (lachrymal) ducts. The definite history of yaws preceding the appearance of the tumours is also of interest, and the fact that nasal discharge was not frequent till after the onset of growth of the tumours.

To what the origin of the third tumour is due I am doubtful. It is in a position which would lead one to suspect the cause might lie in the Antrum of Highmore, but there is neither apparent thinning of bone, discharge from the left nostril, missing or decayed teeth, nor depression of the left palate. I could get no evidence to support the theory that the nasal passages had been invaded by larvae.

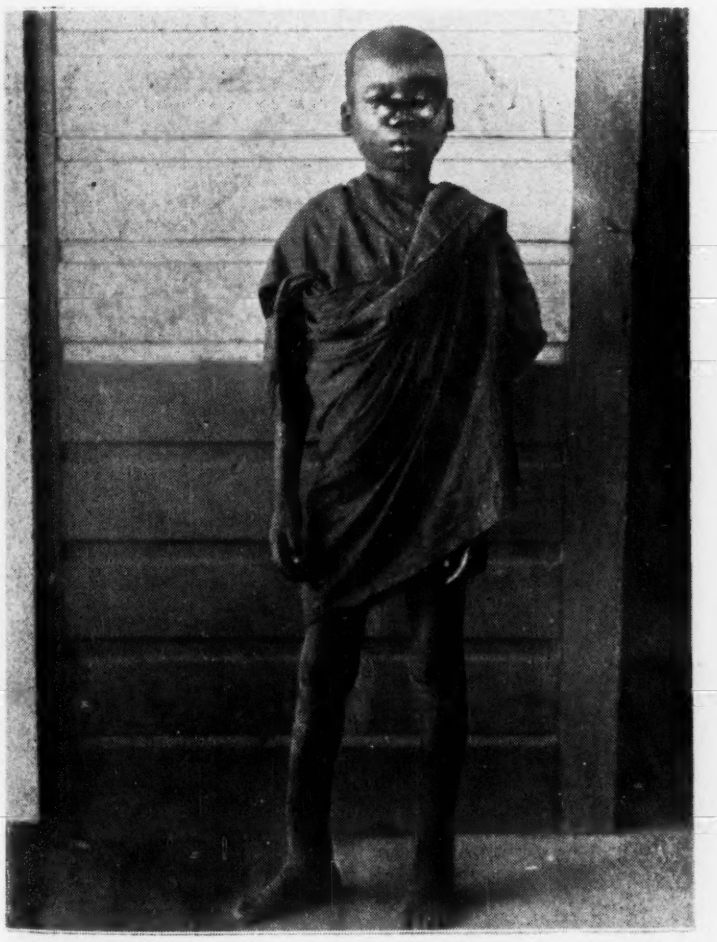
Goundou has been already reported from Sierra Leone, but I believe the disease may be said to be rare in this part of West Africa. I regret the patient refused to allow me to operate for the removal of any of the tumours.



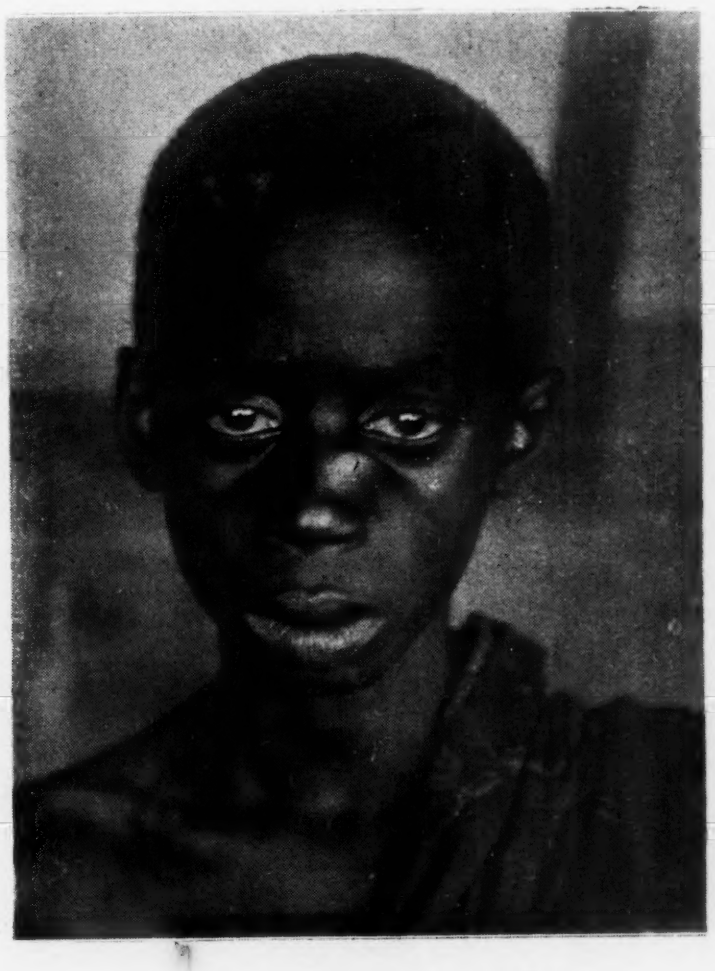
Goundou. Dr. ORPEN'S case.



Goundou. Dr. ORPEN'S case.



Bilateral Goundou, shewing curved tibiae. Dr. KENNAN.



Unilateral Goundou. Dr. KENNAN.

SUB-DRAINAGE AS APPLIED TO THE ANTI-MALARIAL CAMPAIGN ON THE ISTHMUS OF PANAMA

BY

HENRY SIMMS

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(Received for publication 30 *October*, 1908*)*

With the growing interest at present taken in anti-malarial work, and in view of the large works being at present carried out as a result of a fuller realisation of its commercial value, it is believed that the value of any information on the results of the practical application of successful methods not in general use is sufficient warrant for this article. It must be remembered that the methods described are the outcome of experience with the somewhat unusual tropical climate and topography prevailing on the Isthmus of Panama. The rainfall is very heavy, averaging about 180 inches on the Atlantic coast and 80 on the Pacific. The rainy season lasts from May to December, inclusive.

Ordinary methods in the design of sub-drainage for agricultural purposes are not applicable. For the agriculturist the object aimed at is the removal of subsoil water. For Anopheline extermination the object is to remove all water from the surface, and all puddles formed by seepage water, however small. All wet places should become thoroughly dry at least once every ten days. Here this must be accomplished in the face of continuous rains. An engineer without a knowledge of the habits of the Anophelines in question (in this case *Cellia albimanus*, *C. argyritarsis*, and *C. tarsimaculata*) would not be well equipped for the work. The results obtained by the sub-drainage work are shown by its effect on the fever rate. This rate shows the number of cases sent to the hospitals monthly as a percentage of the number of employees appearing on the rolls of the Commission.

At the start of work on the Canal, in the spring of 1904, all the energies of the Sanitary Department were directed towards the suppression of yellow fever and to the cleaning of the two ports of

Panama and Colon, the keys to the health of the Isthmus apart from malaria. Yellow fever was stamped out towards the end of 1905, and the attention of the Department was directed to the reduction of malaria. The conditions prevailing were very favourable to the propagation of this disease. What drainage had been done had been directed towards getting rid of the larger bodies of water. Vegetation was rank, and grew close up to the dwelling-quarters. Alongside the camps, consisting of unscreened houses, were native settlements, 78 per cent. of the population of which showed malaria as a result of blood examinations in fresh specimens, mostly taken from adult men. The place was swarming with Anophelines. White men direct from the North, as well as negroes, were placed in the camps. The negroes spent their evenings in the native villages. In December, 1905, of the total employees 9·63 per cent. were in the hospitals with malaria.

Systematic measures were immediately started. The general scheme was to cut all vegetation growing on soft or soggy places; to confine all water in small surface ditches, and to make a copious use of crude oil. To this was soon added the screening of buildings and cutting of all vegetation for a distance of six or seven hundred feet from the houses, which distance seemed to comprise the length of flight of the Anophelines. Quinine also played an important part. At first the labouring employees would not take the drug, but they had no objection to the more palatable mixture of quinine and rum subsequently tried. This mixture, giving 4 grains of quinine to the wineglass, was made with an inviting colour, and was very successful.

The maintenance of the open ditch system, involving re-grading, filling holes scoured out in heavy rains, removal of vegetation and algae, application of larvicides, the fact of the collapsing of banks and continual inspection, were so expensive, besides being unsatisfactory, that the authorities found it necessary to use some more permanent methods involving less annual expense in upkeep. They recognised that while there was water within reach of mosquitoes there would be larvae, as experience had shown that with the greatest care in the use of larvicides, and with the best inspection, larvae would escape destruction. They reasoned that the only practical method, in view of maintenance costs, was to get rid of the water. Sub-drainage was considered, and a first shipment of tile was ordered and installed.

The success of this was so complete that extended sub-drainage was decided on.

Tile drainage work was started early in 1906, but it was not until towards the end of the year that sufficient was laid to begin to show its effects. During 1907 the work was vigorously conducted. The figures given below will show the effect produced. It must be stated that the figures given for 1906 do not fairly express the conditions. In some districts hospitals had not yet been erected; also the coloured employees had not yet realised the value of the hospitals, and would lie about the villages and camps. It was not until a systematic inspection was started whereby the District Physician and Sanitary Inspector would visit the houses and camps, sending all cases to the hospitals, that true statistics became available. The month of March is selected as being towards the end of the dry season, and August as the period during which the fever rate reaches the highest point. The figures show the percentage of employees passing through the hospital during the month. The third column shows the annual average per 1,000 of deaths from malaria, based on the number of deaths in August for the years shown:—

	March.	August.	Annual Average per 1000 of Deaths.
1906	6·32	9·01	10·55
1907	4·16	5·33	5·05
1908	1·23	3·43	2·44

As a rule, the breeding areas giving the greatest trouble here, besides small streams, swamps, and low-lying, soggy land, are seeps on the sides and at the bases of hills from water following small areas of impervious strata. At Ancon this is specially marked. They are difficult to find, and the smallest depression holding water can breed enough Anophelines to send up the rate for a given camp.

The tile used was of three sizes: 4″, 6″, and 10″, porous and unglazed. Where the tiles had to receive seepage water only, they could be laid at grades as low as one-half per cent., care being taken to place a wisp of grass over the joints. If the soil was very

bad the pipe could be surrounded, and covered for about four inches, with coarse gravel or crushed rock, and the trench filled with earth other than clay. It is necessary to make systematic borings with a 6″ augur to determine the direction of flow in the soil. Here this often follows an almost vertical direction, causing the trenches to be very close or very deep. As a rule, however, the main tile follows the course of a small stream, with feeders coming in from the side seeps. Here the problem is to lay a pipe, as a general rule through stiff clay, so that a considerable torrent can pass over it, and such that a few hours after it will remove every drop of water and suffer no damage from erosion. Such a pipe has of necessity to pass silt in quantities during freshets. Experience has shown that a 6″ pipe will satisfactorily pass silt at a grade of one per cent. and a 10″ pipe at a grade of one-half per cent. These grades can be made 'flatter' if a head can be obtained to act on the pipe, but should be avoided if it is possible. Should 'flatter' grades be required, concrete ditches or stone ditches set in cement mortar should be used.

In starting operations on a valley, the first thing to study is the best method of straightening the stream with a view to getting greater fall and a shorter distance. If the grade exceeds 5 per cent., small waterfalls should be introduced to break the velocity over the surface, during floods. The pipes should be laid at least 2′ 6″ below the bed of the stream, the trenches should be carefully graded with an instrument and the pipes laid evenly and true, with joints open to the extent of $\frac{1}{8}$″ to $\frac{1}{4}$″. They should be firmly imbedded in crushed rock on all sides, extending at least 4″ above the tile. Rock, broken to about 4″ cubes, should then be filled in, up to near the surface, and the last layer finished off in heavy stone if the scour is great. Some small stone must be placed at the top to prevent a too free entry of silt in the first rains. All side branches should be treated in the same way, and should connect to the main with Y junctions. When surface water has to run in volume over the pipe, the outfall should be carefully planned so as to pass the water away quickly, and should be strong enough to stand the scour that occurs at this point.

It would appear probable that after a short time the porous stone placed over the pipe would completely clog, but this does not occur. What happens is that the first heavy rains completely cover the stone with about 2″ of coarse granular earth. The grass quickly

grows over this and forms a complete mat over which the rain runs without erosion. The air from below keeps this mat very porous, and the mat then acts as a filter for future rain. Care must be taken to cut down any trees the roots of which will interfere with the pipes.

In the case of seeps, if the borings made do not give sufficient data, two or more holes must be dug and the slope that the water is following determined. This is sometimes so steep that parallel pipes have been laid, 10′ apart and 5′ deep, to catch completely the water, as it is essential that all water should be intercepted.

With regard to cost, it must be remembered that the cost of transportation and labour is exceedingly high on the Isthmus. A good labourer receives $1.80 per day U.S. currency. Some work would be in inaccessible valleys, and complete roads would have to be built. Rock would often have to be transported by train and then carried over hills. This transportation has made the work considerably more costly than it would be in other places. The average cost of the tile put in so far is about 35 cents per foot, about 1s. 3d. of which is the first cost of the tile. The cost of maintenance of open ditches for the year amounted to 25 cents per foot, so that in less than a year and a half the work has paid for itself, apart from the great reduction in malaria and the great saving in the cost of the care of the sick. The work is yet far from complete, and much remains to be done.

From the experience gained here it would appear that sub-drainage work is the only practical means, taking into consideration the cost of upkeep, for permanent anti-malarial work. In a climate such as this, and where Anophelines breed in clear running water during all months of the year, the annual expense of the open ditch system is prohibitive.

A NEW CULICID GENUS

BY
F. V. THEOBALD, M.A.

(Received for publication 6 November, 1908)

GENUS *Newsteadina*, nov. gen.

Near *Orthopodomyia* Theobald, but differs in the longer male palpi, and the presence of very long scales on the male antennae, and also in the wings having *Mansonia*-like scales.

Head clothed with dense, narrow-curved scales, somewhat broadened, numerous upright forked scales broadly expanded apically and with flat lateral scales.

Antennae plumose in the ♂, the basal segments with very long, narrow, twisted, or wavy scales; pilose in the ♀, with narrow outstanding flat scales on the two basal segments. Palpi of the ♂ of four segments (?), as long as the proboscis, apical segment very small; of the ♀ longer than half the proboscis.

Thorax with rather long, narrow-curved scales; scutellum with rather broad, curved scales; metanotum nude.

Wings clothed along the veins with large, asymmetrical, flat scales (*Mansonia* type), and thin, straight, lateral ones beneath them. Scaled black and white, giving the wings a markedly ornamental appearance.

TYPES. *aboricollis*. D'Emerrez de Charmoy. Ann. Trop. Med. and Parasit.* Vol. II, No. 3, p. 257, July, 1900. In the museum of the School of Tropical Medicine, Liverpool University.

Culex fowleri (loc. cit.) The insect described under this name is, judging from the single male, a *Grabhamia*.

Culex rolandi (loc. cit.) is undoubtedly only *Culex micro-annulatus*, Theo.

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* In the description of this insect in the journal referred to, the following note occurred:—'This remarkable insect bears some resemblance to Theobald's genus *Lophoceratomyia*, though it is quite distinct, and a new genus will probably be erected to receive it.—R. NEWSTEAD.'

THE INFLICTED TALIPES OF THE CHINESE

BY

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LATE DEMONSTRATOR OF ANATOMY IN THE UNIVERSITY

(Received for publication 14 *November*, 1908*)*

Actual specimens of the feet of Chinese women are sufficiently rare in this country to warrant the following remarks, which are relative to a foot kindly presented to the Museum of the Liverpool Tropical School of Medicine by Dr. J. Bell, Hong Kong. Since there are in the Museum of the College of Surgeons of England, and elsewhere, fully dissected specimens, it was considered advisable to keep this one in its entirety. It consists of a left foot and the lower end of the leg, the section having been made straight across the limb, $1\frac{3}{4}$ inches above the inner malleolus. The age of the subject is unknown, but it is certainly the foot of an adult woman. The following are some of the more important measurements:—

Total length, $5\frac{7}{8}$ inches.

Breadth at metatarso-phalangeal joint, 3 inches.

Height from the ground to upper surface of scaphoid, 3 inches.

Maximum height from the ground to lower surface of soft tissues, $\frac{3}{4}$ inch.

Distance between the supporting points, 3 inches.

The most striking point at first sight is that the fifth toe is little more than half the distance from the heel to the tip of the great toe. This description conveys, more than actual measurements, the degree of antero-posterior shortening present. But it may be mentioned, for the sake of comparison, that normally the fifth toe is situated at a distance from the heel of eight-elevenths of the total measurement.

The position is, roughly, in surgical terms one of Pes Cavus, accompanied by a varoid deformity of the outer three toes, and a valgoid position of the inner two. The transverse creases often seen in talipes acquired from any cause are plainly visible. They run horizontally behind the Tendo Achillis, above the heel. The deep furrow across the sole divides the foot as a supporting structure into

two portions—an anterior, triangular, with the base backwards and measuring 3 inches from front to back, and a posterior, circular, measuring 2 inches in diameter.

X-ray photographs were kindly taken by Mr. C. Thurston Holland, radiographer to the Royal Infirmary, and these throw much light on the nature of the deformity and the probable mechanism of its production. In the skiagram taken from above, the great toe is seen to be in a valgoid position, the greater amount of dislocation being at the metatarso-phalangeal joint. This outward position is not more than that of the great toes of many European civilized women. The second and third toes show the same change at the metatarso-phalangeal articulations, whilst the interphalangeal joints are acutely flexed. In the fourth and fifth toes this flexion of the interphalangeal joints completely masks the fact that the first phalanges are pushed outwards from their metatarsal bones.

The first impression, on looking at the side view, is that the posterior part of the os calcis is in line with the tibia instead of being at right angles to it. Indeed, a radiograph of the os calcis alone, when compared with that of a normal bone, explains approximately half of the extremely arched condition of the foot. The posterior half of the os calcis is set almost at right angles to the anterior half. How this alteration in the shape of the calcaneum has been brought about is open to question. There are two possible explanations, either of which may be sufficient. A consideration of the ossification of the bone and of the accompanying radiograph supports the view which I take, that the change is mainly in the soft, newly-formed portion of the os calcis which is in contact with the so-called epiphyseal line (a better term for which would be epiphyseal plane), or perhaps in the epiphyseal plane itself. The cartilaginous calcaneum at birth contains a small central nucleus of bone which ultimately forms the main portion. At about the tenth year a speck of bone appears at the posterior end, and these bony formations meet at the sixteenth year. According to my view, the pressure, which is as a matter of fact applied in an antero-posterior direction, has caused the epiphyseal end to slide, as it were, round the main body of the bone until it occupies a position below instead of behind it. Against this view it may be argued that the epiphysis forms a relatively small proportion of the bone, as evidenced by the thin flake of bone which

can just be separated from the rest of the calcaneum at about the sixteenth year, the growth of bone having been actively taking place for only six years. But every epiphyseal plane is a travelling plane, progressing in a direction away from the centre to the end of the bone, and originally this plane must have occupied a position much further forward, possibly as far as the point *x*.

Another view which occurs to me, but which I regard as less likely, is that by the force of the bandages added to that of the Tendo Achillis the epiphysis, though forming new bone, cannot form it in an antero-posterior direction, and thus perform its true function of adding to the length of the bone. It is therefore compelled to deposit ossific material in the direction of least resistance, namely, downwards, and this downward prolongation is therefore somewhat of the nature of an exostosis. The upward pressure of the ground, corresponding to the weight of the child, must on this assumption offer less resistance than the bandages.

In the X-ray photographs it will be noted that, according to the former view, the original posterior or epiphyseal end of the calcaneum is from *y* to *z*, and the patient walks on the insertion of the Tendo Achillis, a statement made in 1880, and based on a dissected specimen.

According to the latter explanation, the original posterior end of the os calcis is from *x* to *z*, and the Tendo Achillis is still inserted about half an inch below the point *x*.

The os calcis, then, is in any case responsible for the posterior half of the arch. In contrast to this, the anterior half is produced by a small change in several bones, rather than by a great change in one. From the summit of the arch (the astragalus) forwards, there is a slight degree of shifting of the articular surfaces of each bone. This is a purely anatomical change, and it is doubtful if any particular notice would be taken of these individual bones by any anatomist.

The photographs are of value clinically in that they illustrate, to the naked eye at all events, the emphatic statement made long ago by Hilton that the fixation of a healthy joint, even for years, produces no pathological change. They are, further, of interest to the orthopaedic surgeon, in that they shew how much can be done by manipulation and continued pressure.

In the production of the deformity, which takes place during the fifth to the seventh years, the size of the foot is reduced by bandages which turn the outer toes inwards and under the sole to make the foot narrow, while the bandages applied from front to back make it short.

The specimen is regarded by those familiar with the East in years gone by as a not excessive instance of the practice. The evidence of ulceration seen on the skin is in its slightness quite the exception to the rule, gangrene being of fairly common occurrence with the frequent loss of one or more of the outer toes.

DESCRIPTION OF PLATES

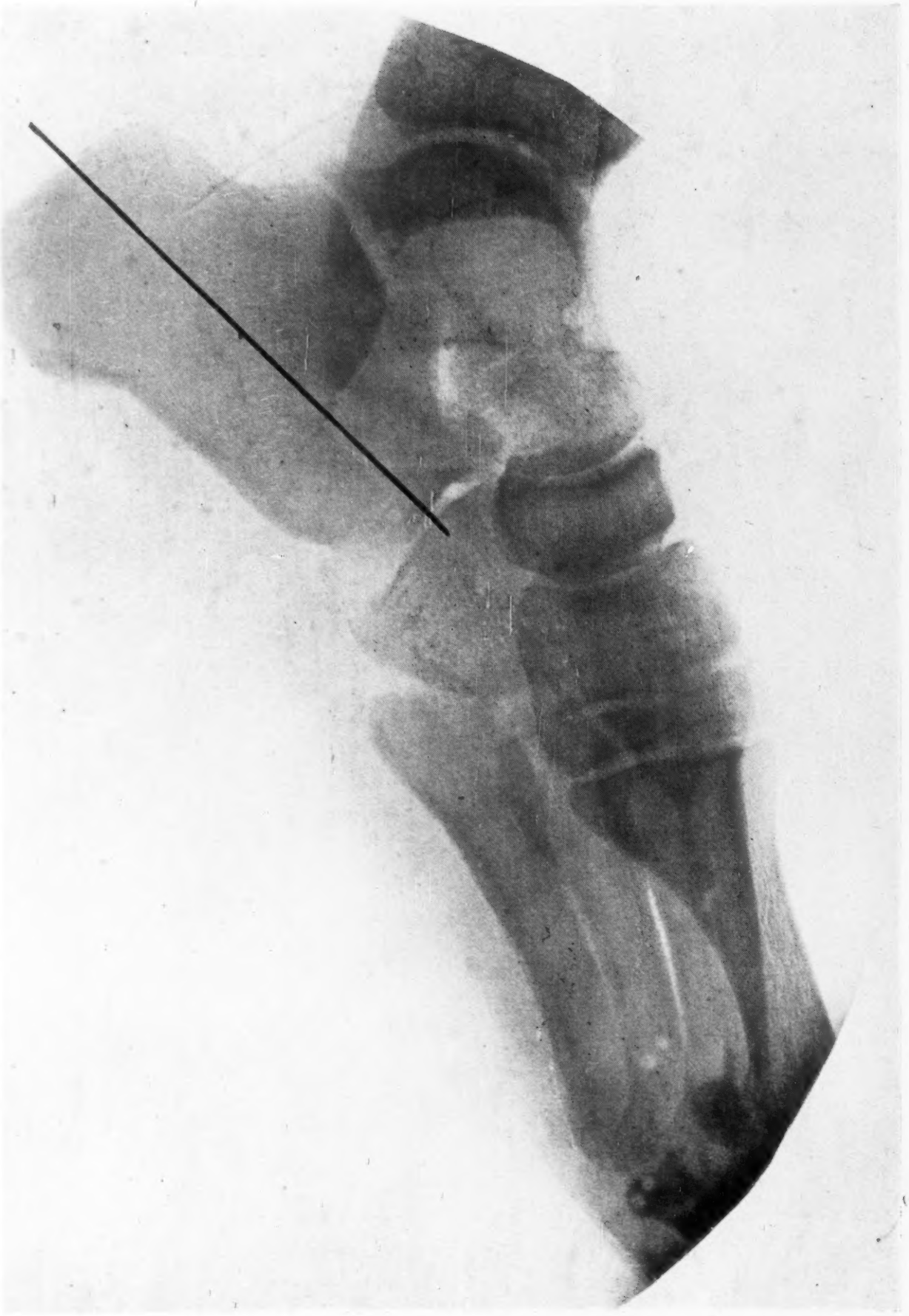
I—X-ray of normal foot from the side.

II—X-ray of Chinese foot from the side.

A = axis of anterior half of os calcis.
P = axis of posterior half of os calcis

III—X-ray of Chinese foot from above.

IV, V—Foot from side and from below.



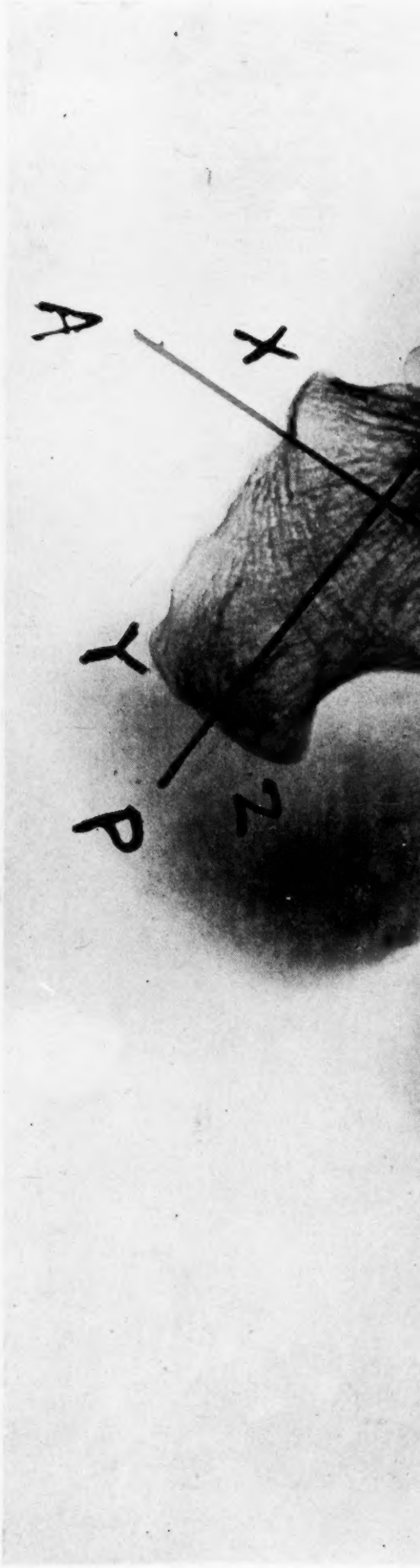
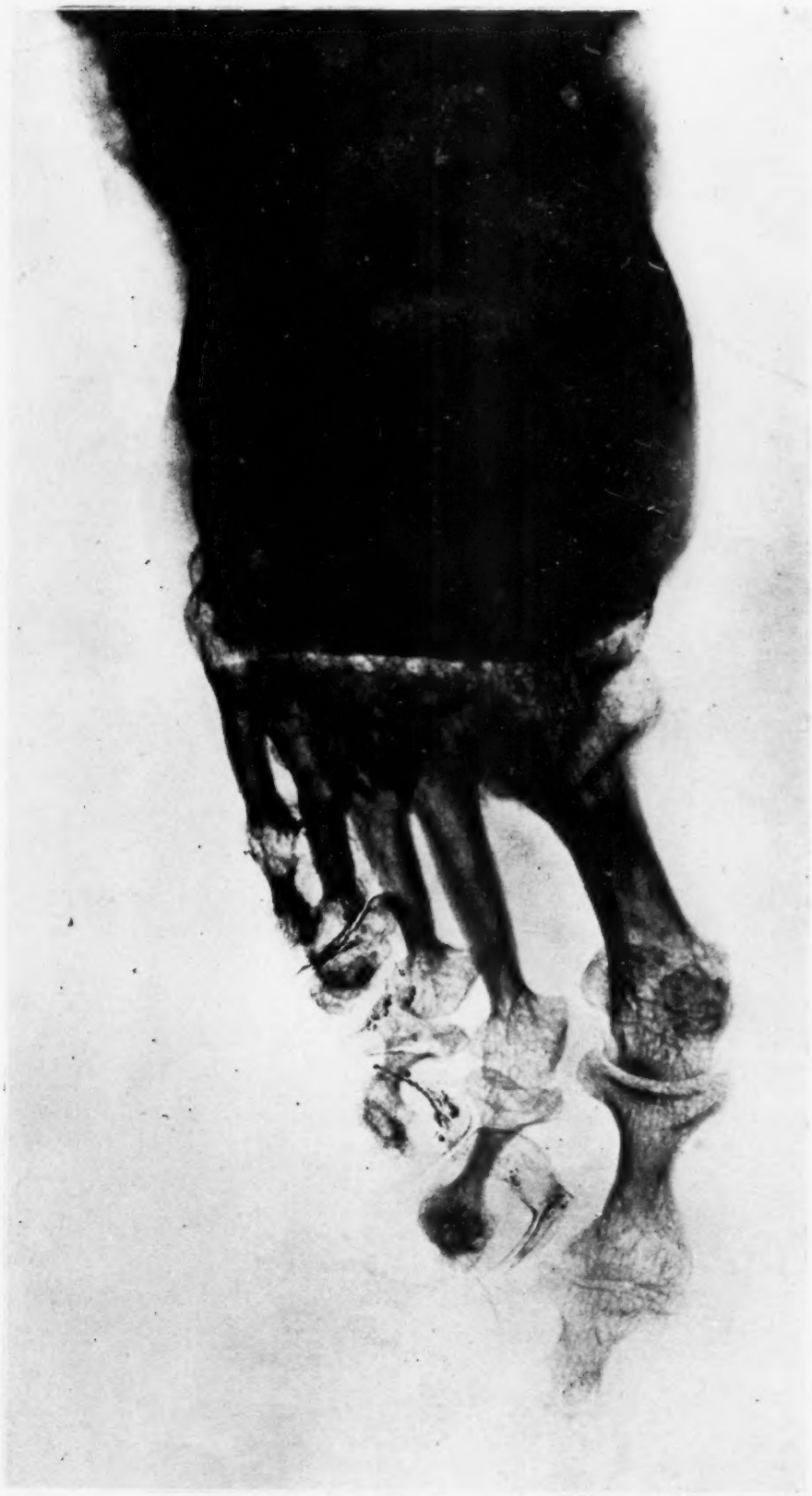
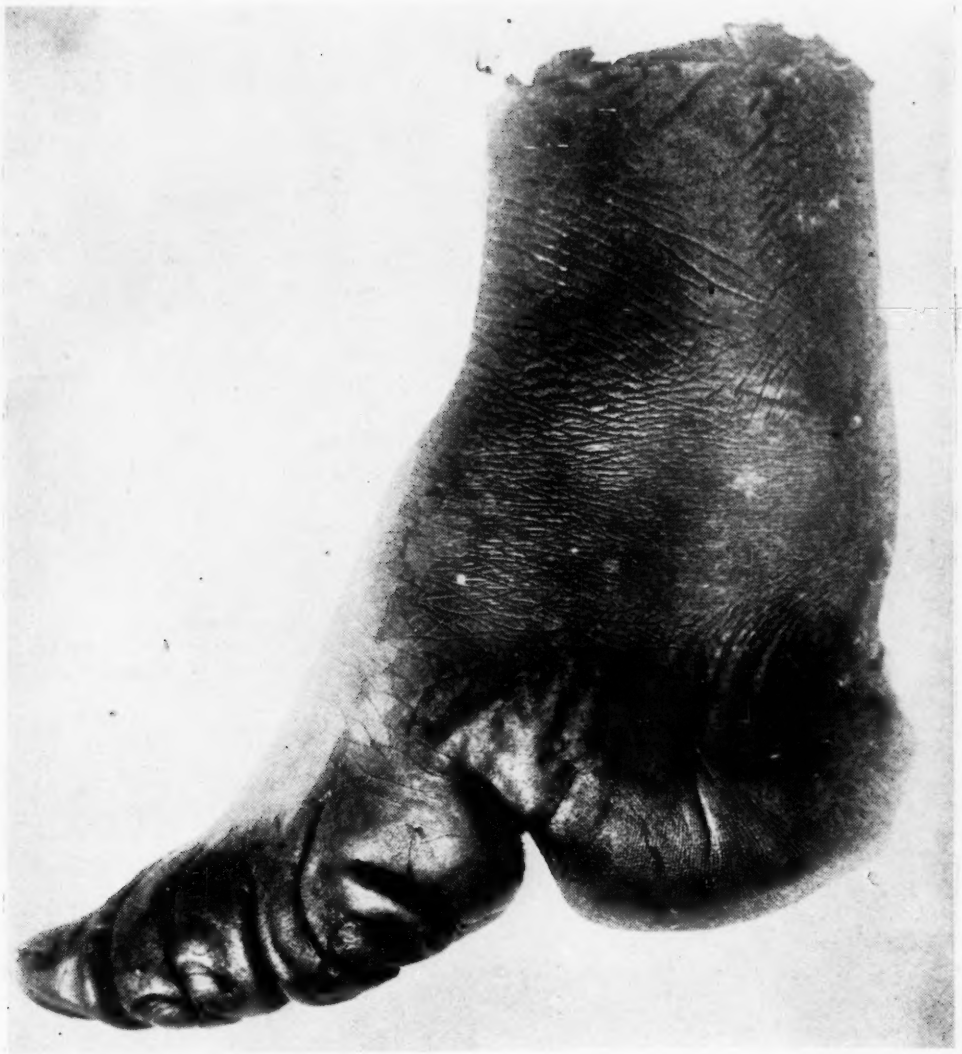
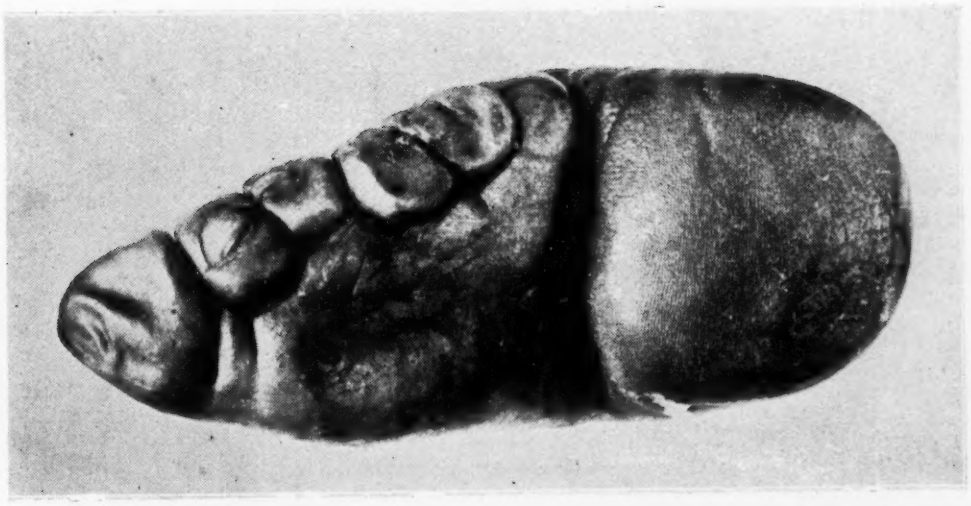

A
X
Y
P

PLATE II

PLATE III







CONTRIBUTION A L'ETUDE DE *POROCEPHALUS MONILIFORMIS*

PAR
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DIRECTEUR DU LABORATOIRE
ET
J. RODHAIN
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Laboratoire de Léopoldville (Congo belge)

(Received for publication 16 November, 1908)

Dans une communication précédente, publiée dans ces *Annals*, Vol. I, No. 4, 1908, nous avons relaté quelques cas d'infection naturelle par larves de *Porocephalus moniliformis*, Dies; une observation chez le nègre, quatre observations chez des singes.

Nous avons pu depuis lors constater d'autres cas d'infection naturelle par ce parasite chez le singe et le serpent. Nous avons ensuite par une série d'infections expérimentales, élucidé complètement le cycle évolutif de *Porocephalus moniliformis*.

I. INFECTIONS NATURELLES

Malgré les autopsies assez nombreuses de nègres originaires de différents points du Congo, nous n'avons plus constaté d'infection par larves de *Porocephalus*. Comme nous le disions dans notre précédente communication, ces infections chez l'homme sont donc extrêmement rares.

Nous avons été plus heureux chez les animaux, et depuis notre première publication, 5 singes et 3 serpents furent trouvés infectés.

A. SINGES.

14.X.07: un petit singe *Macacus* meurt dans la journée; à l'autopsie nous trouvons 2 larves de *Porocephalus* encapsulées dans l'épiploon.

14.III.08: un singe *Macacus* est trouvé mort: dans l'épiploon il y a 6 larves de *Porocephalus*; pas une seule dans les organes.

10.IV.08 : petit singe *Macacus*, mort dans la journée ; dans l'épiploon 2 larves de *Porocephalus* ; organes indemnes.

25.IV.08 : singe *Macacus* mourant, est autopsié immédiatement. Dans la cavité péritonéale, nous trouvons 1 larve de *Porocephalus* libre ; dans le grand épiploon, 2 larves presque complètement sorties du kyste, 1 larve encore bien encapsulée. Pas d'éxsudat dans le sac péritonéal, pas de lésions organiques. Les larves mesurent environ 15 mm. après fixation ; ce sont 4 ♀.

27.V.08 : singe *Macacus* est trouvé mort au matin. Dans le grand épiploon il y a 4 larves de *Porocephalus* ; dans le mésentère, 1 larve ; toutes sont bien encapsulées. Pas de lésions organiques.

Ces 5 singes, comme les 4 précédents dont nous avons fait mention dans la précédente publication, avaient été achetés à Léopoldville à des nègres, qui les avaient amenés du Haut-Congo. Il nous fut impossible de déterminer plus exactement le lieu d'origine.

La disposition des larves de *Porocephalus* était identique chez tous les singes : nous l'avons décrite dans notre première note.*

Les caractères morphologiques étaient les mêmes que ceux indiqués dans cette même notice, pour la larve du nègre.

Nous attirons dès maintenant l'attention sur la grande fréquence de l'infection naturelle par *Porocephalus* chez les singes *Macacus* au Congo. Sur 31 singes que nous avons eus à notre disposition depuis février 07 à mai 08, 9 furent trouvés infectés.

Dans l'étude que nous ferons plus loin de l'infection expérimentale, nous verrons que ces singes ne se sont pas infestés à notre Laboratoire, hypothèse que nous avions émise dans notre première communication.

B. Serpents.

A Léopoldville les grands serpents sont relativement rares. Nous avons pu en examiner 4, dont 3 renfermaient des *Porocephalus* adultes dans les sacs pulmonaires.

7.XII.07 : serpent No. 1, *Bitis gabonica*,† Sué à Léopoldville. Renferme dans le sac pulmonaire 29 vers *Porocephalus* de dimensions variables, et 3 vers dans le tissu conjonctif avoisinant les poumons.

* Ces Annals, Vol. I, No. 4, février 1908.

† Nous devons la détermination des différentes espèces de serpents dont il sera question dans cette note, à l'obligeance de Mr. G. A. Boulenger, du British Museum. Nous l'en remercions vivement.

Ces derniers vers sont de dimensions plus petites que ceux renfermés dans le poumon. Il y avait 15 ♂ et 15 ♀, 2 vers furent detruits.

27.IV.08 : serpent No. 2, *Python sebae* : † nous trouvons dans les sacs pulmonaires 13 vers *Porocephalus* (8 ♀, 5 ♂), identiques aux précédents.

23.VII.08 : serpent No. 3, *Python sebae* : † renferme dans les sacs pulmonaire 17 *Porocephalus* (10 ♂, 6 ♀, 1 en très mauvais état).

Caractères des Parasites. Ces *Porocephalus* ont une teinte jaune pâle, sont de longueur variable ; les ♂ étant sensiblement plus petits que les ♀, et présentant les caractères généraux décrits pour ces parasites. Le nombre des segments est en général de 20 pour les ♀, et de 17 ou 18 pour les ♂.

Les *œufs*, sont arrondis, incolores. A l'intérieur d'une première enveloppe assez mince, se trouve l'œuf ou l'embryon, entouré d'une membrane épaisse à double contour, et n'occupant en général que les $\frac{2}{3}$ ou les $\frac{3}{4}$ de l'espace limité par l'enveloppe extérieure. Ce qui caractérise surtout l'embryon, c'est l'existence de 4 crochets bifurqués, paraissant terminer autant d'articles ou de pattes, disposés symétriquement.

Pour obtenir les œufs en quantité suffisante pour nos essais d'infection expérimentale nous avons gratté les parois des poumons chez les serpents infestés, ou dilacéré et exprimé un ver femelle.

En parcourant la littérature se rapportant aux *Porocephalus*, il nous a semblé qu'il régnait une confusion assez grande dans la dénomination des différentes espèces. L'étude de nos parasites au point de vue morphologique et histologique, leur détermination exacte par comparaison avec d'autres types, nécessitant un temps assez considérable, nous n'avons pu la faire. Monsieur Gedoelst, professeur de parasitologie à l'Ecole de Médecine vétérinaire à Bruxelles, a bien voulu se charger de ce travail.

II. INFECTION EXPÉRIMENTALE

Pour élucider le cycle évolutif du *Porocephalus moniliformis*, soupçonné mais non encore étudié, il fallait en premier lieu rechercher le mode d'infestation de l'hôte ou des hôtes intermédiaires, ensuite réussir l'infection de l'hôte définitif, le serpent.

En effet, toutes les observations recueillies jusqu'à présent tant

chez l'homme que chez les animaux prouvent que le *Porocephalus moniliformis* ne peut arriver à l'état adulte que dans les poumons de certains serpents. L'homme et les autres animaux ne constituent que les hôtes intermédiaires.

(a) Infection de l'hôte intermédiaire.

Après les observations d'infection naturelle recueillies par différents observateurs et nous-mêmes, il paraissait à peu près certain que l'animal s'infestait* eu avalant accidentellement des œufs de *Porocephalus.*

Nous avons donc recueilli chez les 3 serpents infestés, un nombre considérable d'œufs mis en suspension dans de l'eau physiologique. Cette suspension était ensuite mélangée prudemment à du biscuit ou de la viande, servant à l'alimentation de nos animaux.

1° *Singes.*

Singe No 1, Macacus: le 7.XII.07, est infesté avec des œufs de *Porocephalus* provenant du serpent No. 1 *(Bitis gabonica)*; il meurt accidentellement le 21.II.08, ou le 77e jour après l'infection. A l'autopsie, nous trouvons le grand épiploon farci de jeunes vers *Porocephalus*, tous encapsulés. Il n'y a pas de vers libres dans la cavité péritonéale, pas de vers dans les organes abdominaux. Dans la cage thoracique, il y a 2 vers très petits, enkystés à la surface du poumon droit.—Ces larves ne montrent encore ni crochets, ni orifices sexuels.

Singe No 2, Macacus: le 7.XII.07, est infesté avec des œufs de *Porocephalus* du serpent No. 1 *(Bitis gabonica).* Ce singe très vif a été gardé en observation durant plus de 5 mois et ne s'est jamais montré malade. Il fut tué le 15.V.08, c'est-à-dire le 161me jour après l'infection.

A l'autopsie, le grand épiploon renferme une quantité extrêmement considérable de jeunes vers *Porocephalus*, tous encapsulés; la presque totalité des vers se recontre le long des bords libres de l'épiploon.

Le mésentère renferme de nombreux vers enkystés.

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* Nous n'avons pu prendre connaissance, qu'après notre retour en Belgique, de l'important travail de Ch. W. Stiles, Bau u. Entwickelungsgeschichte von *Pentastomum proboscideum*, Rud., u. *Pentastomum subcylindricum*, Dies., in *Zeitsch. f. wissenschaft. Zoologie*, LII, 1891.

Les ganglions lymphatiques mésentériques sont hypertrophiés et renferment plusieurs vers encapsulés, à divers stages de développement.

Le foie, à sa face supéro-antérieure, présente 5 vers, très petits, encapsulés ; à sa face inféro-postérieure, 3 vers. Dans le parenchyme de l'organe, il n'y a pas un seul parasite.

La rate est un peu hypertrophiée, mais ne renferme pas de ver.

Le diaphragme, à sa face supérieure comme à sa face inférieure, présente plusieurs vers.

Les reins ne renferment pas de vers.

L'estomac présente un ver encapsulé, à sa face externe, le long de la grande courbure.

L'intestin grêle, à l'union du tiers antérieur avec le tiers moyen, présente 1 ver encapsulé dans la sous-muqueuse. Dans le contenu intestinal nous n'avons pas trouvé un seul ver.

Il y a une dizaine de vers encapsulés à la face interne de la paroi abdominale et du petit bassin.

Dans la cage thoracique, le poumon gauche est indemne ;—le poumon droit, à sa surface, présente 3 vers encapsulés, 1 sur le lobe supérieur, 2 sur le lobe inférieur.

Le péricarde porte 1 ver enkysté à sa face antérieure.

Un certain nombre de parasites, les plus développés, ont déjà des petits crochets et des orifices sexuels. D'autres, moins développés, n'ont pas encore ces appendices.

Singe No. 3, Macacus, est infesté le 7.III.08, avec des œufs de *Porocephalus* du serpent No. 1 *(Bitis gabonica)*, gardés dans de l'eau physiologique, depuis le 7.XII.07, c'est-à-dire depuis 3 mois. Il est tué le 18.III.08 ou le 12me jour. A l'autopsie, malgré un examen minutieux, nous ne trouvons pas trace de lésions indiquant un commencement de développement des œufs.

Singe No. 4, Macacus, est infesté le 7.III.08, avec des œufs de *Porocephalus* du serpent No. 1, gardés dans de la terre depuis le 7.XII.07, c'est-à-dire depuis 3 mois. Les deux premiers mois, la terre fut gardée humide, le troisième mois elle fut négligée, et resta plutôt sèche.—Ce singe est encore en vie. (1.VIII.08.)

Singe No. 5, Macacus, est infesté le 27.IV.08, avec des œufs de *Porocephalus* du serpent No. 2 *(Python sebæ)*. Est encore en vie (1.VIII.08).

2° *Rats.*

Le 6.VI.08, 5 rats gris indigènes, sont infestés avec des œufs de *Porocephalus* du serpent No. 2, et gardés dans de l'eau physiologique depuis le 27.IV.08.

Rat No. 1, tué le 26.VI.08, ou le 21me jour, pas infecté.

Rat No. 2, tué le 11.VII.08, ou le 36me jour, pas infecté.

Rat No. 3, trouvé mort le 17.VII.08, et à moitié dévoré par les deux survivants.

Rat No. 4 (Mus rattus), tué el 27.VII.08, ou le 52me jour, est infecté. A l'autopsie, nous trouvons dans le grand épiploon de nombreuses granulations, grosses comme une petite tête d'épingle. L'examen microscopique montre dans ces granulations la présence d'un tout petit ver vivant. Le mésentère présente également de nombreuses granulations. La rate et les reins, portent à leur surface quelques granulations identiques ; il n'y en a pas une seule à l'intérieur de ces organes.

Les autres organes sont indemnes.

Rat No. 5 (Mus rattus), tout jeune, tué le 27.VII.08, out le 52me jour. A l'autopsie, nous constatons des lésions identiques à celles du rat No. 4 : les mêmes organes sont infestés, mais on outre il y a de très rares granulations dans les deux poumons.

3° *Coq.*

Un jeune coq fut infesté le 27.IV.08 avec des œufs de *Porocephalus* du serpent No. 2 ; il fut tué le 15.VI.08 out le 50me jour, mais n'avait pas contracté d'infection.

4° *Canard.*

Un jeune canard ♂ fut infesté le 27.IV.08 avec des œufs de *Porocephalus* du serpent No. 2 ; il fut tué le 26.V.08 ou le 30me jour, mais n'avait pas contracté l'infection.

5° *Chacal.*

Un jeune chacal ♀ fut infesté le 7.XII.07 avec des œufs de *Porocephalus* du serpent No. 1 ; il fut tué le 10.IV.08 ou 95me jour, mais n'avait pas contracté d'infection.

6° *Chat.*

Un jeune chat sauvage ♀ fut infesté le 8.V.08 avec des œufs de *Porocephalus* du serpent No. 2, gardés dans de l'eau physiologique

depuis le 27.IV.08. Il fut trouvé mourant le 28.V.08, ou le 21me jour, et autopsié immédiatement.

Dans le grand épiploon et le mésentère, nous remarquons quelques petits points blancs, comme une petite pointe d'épingle. Au microscope, ces petits nodules sont composés d'une partie centrale ou l'œuf de *Porocephalus* plus ou moins modifié, et d'une partie périphérique formée par la réaction cellulaire. Dans certains œufs, nous avons retrouvé encore un ou deux crochets parfaitement reconnaissables.

7° *Homme.*

Deux hommes et une femme furent infestés avec des œufs de *Porocephalus* du serpent No. 2. Disons immédiatement que tous trois étaient arrivés au stade ultime de la trypanosomiase, et que chez aucun d'eux, l'ingestion d'œufs de *Porocephalus* ne provoqua le moindre symptôme pathologique.

(*a*) *John* ♂, arrivé à la période ultime de la trypanosomiase, complètement fou ; il s'était refusé énergiquement et obstinément à tout traitement.—Le 27.IV.08, il avalé quelques centimètres cubes d'eau contenant des œufs de *Porocephalus* du serpent No. 2. Ultérieurement il n'a pas présenté le moindre symptôme morbide pouvant être attribué aux *Porocephalus*. Il meurt de trypanosomiase le 30.VI.08, c'est-à-dire le 65me jour après avoir avalé les œufs.

Autopsie: pas de lésions cutanées, nutrition assez bien conservée.

Abdomen: dans la cavité péritoneale, pas d'exsudat, guère de traces de lésions inflammatoires.

Grand épiploon: dans sa partie supérieure présente d'innombrables tâches blanches, opaques, aplaties, ayant jusque 2 mm. de diamètre. Au microscope, ces tâches ne montrent pas d'organisation (œufs de *Porocephalus* résorbés?). Le long des bords libres de l'épiploon, quelques granulations plus ou moins transparentes, grosses comme un demi-grain de riz. Il suffit de les dilacérer légèrement et prudemment pour en faire sortir un petit ver *Porocephalus*.

Intestin grêle, sur toute sa longueur présente à la face externe de nombreuses granulations, dont la plus grosse ne dépasse pas la moitié d'un grain de riz. Quelques-unes ne contiennent pas ou plus de *Porocephalus*, d'autres renferment un jeune ver identique à ceux de l'épiploon. Incisé sur toute son étendue, l'intestin est vidé de son

contenu. La muqueuse présente en de nombreux endroits, des petites tuméfactions produites par des granulations siégeant dans la sous-muqueuse, et renfermant un jeune ver *Porocephalus.*

En examinant méthodiquement le contenu intestinal recueilli dans une cuvette, nous y trouvons 1 *Taenia,* quelques Ankylostomes, puis une vingtaine de petits vers *Porocephalus* ; nous en avons retrouvé encore deux accolés aux replis de la muqueuse.

Gros intestin: ni à la face externe ni à la face interne nous n'avons trouvé de granulations ; la muqueuse présente quelques cicatrices d'ulcérations anciennes.

La *rate* et les *reins* ne présentent rien de spécial. Les nombreuses incisions faites dans ces organes ne révèlent pas de *Porocephalus.*

Foie: n'est pas augmenté. A sa surface, il présente à sa face antérieure comme à sa face postérieure, de nombreuses granulations plus ou moins grosses, la plus volumineuse ne dépassant pas un demi-grain de riz : toutes ces granulations renferment un jeune ver *Porocephalus.* Après incision, nous constatons que la parenchyme hépatique reuferme de nombreuses granulations à ver *Porocephalus.*

Cage thoracique: les 2 poumons présentent à leur surface quelques rares granulations à *Porocephalus* ; dans le tissu pulmonaire 3 granulations identiques.

Ganglions lymphatiques: nous avons examiné 5 ganglions de plexus coeliaque, 2 ganglions du médiastin antérieur, sans trouver de *Porocephalus.*

(*b*) *Wadi* ♂, atteinte de trypanosomiase à la période ultime, devenu aveugle après traitement à l'atoxyl ; absorbe le 27.IV.08, un peu d'eau avec des œufs de *Porocephalus* du serpent No. 2. Depuis ce moment, le malade n'a pas présenté le moindre symptôme morbide pouvant être attribué aux *Porocephalus.* Il meurt le 6.VII.08, c'est-à-dire le 71me jour après l'ingestion des œufs.

Autopsie: les constatations sont identiques à celles faites chez le sujet précédent, *John* : infection du grand épiploon, de la tunique intestinale, du foie et des poumons. En outre nous avons trouvé de nombreux ganglions mésentériques renfermant de 1 à 8 vers *Porocephalus* à différents stades de développement.

Les vers trouvés chez ces 2 sujets étaient très petits, et ne présentaient que 2 à 4 mm. de long.

(*c*) *Gwangwate* ♀, atteinte de trypanosomiase à un stade avancé,

incurable ; ingère le 27.IV.08 de l'eau avec des œufs de *Porocephalus* du serpent No. 2.

Encore en vie le 1.VIII.08.

En récapitulant brièvement ces constatations, nous trouvons que l'infection expérimentale par la bouche au moyen d'œufs de *Porocephalus moniliformis* provenant de serpents (*Bitis gabonica*, *Python sebae*), a réussi chez divers animaux, 2 singes (*Macacus*), 2 rats indigènes (*Mus rattus*), 1 jeune chat sauvage, et chez 2 nègres. Le résultat fut négatif chez le coq, le canard et le chacal.

Nous ferons remarquer ensuite que chez aucun des animaux infectés, nous n'avons pu constater de lésions macroscopiques. Les jeunes vers *Porocephalus* encapsulés, étaient fixés sur la séreuse ou dans le parenchyme des organes, mais autour d'eux il n'y avait pas de réaction inflammatoire. Le *Porocephalus*, jusqu' à un certain stade de développement, parait donc bien peu irritant pour les organes.

Nous signalerons ensuite le développement extrémement lent du ver. Ainsi chez le singe *Macacus* No. 2, tué le 161me jour après l'ingestion des œufs, les vers avaient au maximum 12 mm. de long, la plupart ayant des dimensions moindres.

Enfin, quant aux organes infestés, nous ferons remarquer que chez les divers animaux, singes, rats, chat, pas un seul ver ne fut trouvé à l'intérieur des organes. Toujours les parasites étaient fixés à une séreuse, péritoine ou plèure. Par contre chez l'homme, nous avons trouvé de nombreux vers encapsulés dans le foie et quelques-uns dans les poumons.

De ces essais d'infestation expérimentale nous pouvons conclure que les hôtes intermédiaires s'infestent de *Porocephalus moniliformis*, en avalant accidentellement des œufs provenant de vers adultes logés dans les poumons des grands serpents (*Bitis gabonica*, *Python sebae*). Ces œufs doivent passer par la trachée et être avalés ensuite par le serpent pour arriver à l'extérieur par la voie intestinale.

(b) Infestation de l'hôte définitif.

Pour compléter l'étude du cycle évolutif du *Porocephalus moniliformis*, il nous restait à infester un serpent avec de jeunes parasites provenant d'un hôte intermédiaire infecté expérimentalement.

Il nous paraissait probable que dans la nature, les grands serpents devaient s'infester en avalant un animal quelconque (singe, rat),

porteur de jeunes *Porocephalus*. Dans les conditions où nous nous trouvions, nous ne pouvions songer à opérer avec des serpents boa. L'expérience fut tentée avec des petits serpents.

Le 15.V.08, nous faisons avaler à 2 serpents, *Causus rhombeatus* de jeunes vers *Porocephalus* encapsulés dans le grand épiploon du singe *Macacus* No. 1 (infesté expérimentalement le 7.XII.07 avec des œufs de *Porocephalus* du serpent No. 1 (*Python sebae*), et tué le 161e jour après l'infestation).

L'un des serpents fut trouvé mort le 20.VII.08, et ne put être examiné.

L'autre fut tué le 23.VII.08, ou le 70me jour après l'infestation. A l'autopsie nous trouvons dans le sac pulmonaire 4 vers *Porocephalus*, 2 ♂ et 2 ♀. Ces vers ont une longueur, les ♂ de 2 à 2·5 cm., les ♀ de 3 à 3·5 cm., après fixation. Ils sont en tous points identiques à ceux que nous avons trouvés dans les poumons des grands serpents, mais sont de dimensions notablement moindres.

Ainsi donc, nos prévisions s'étaint réalisées : il est possible d'infester des serpents en leur faisant avaler de jeunes vers *Porocephalus*, et nous pouvons en conclure que dans la nature les grands serpents s'infectent en avalant des animaux infestés de jeunes *Porocephalus*.

CONCLUSIONS

1° Les parasites que nous avons trouvés, comme infection naturelle, chez plusieurs singes (*Macacus*) et trois serpents (1 *Bitis gabonica*, 2 *Python sebae*), ne sont que deux stades différents du même parasite : forme jeune chez le singe, forme adulte chez les serpents.

2° L'homme et divers animaux, singe, rat, chat, s'infestent en avalant accidentellement des œufs du parasite ; ils ne constituent que des hôtes intermédiaires.

3° Les serpents, et surtout les grands serpents, s'infestent en avalant un hôte intermédiaire infecté ; ils constituent l'hôte définitif.

L'étude morphologique et histologique des parasites, qui sera publiée ultérieurement par le professeur Gedoelst, prouvera si nous avons eu raison de considérer, avec Neumann et Looss, le *Porocephalus constrictus* comme la forme jeune du *Porocephalus moniliformis*.

LITTÉRATURE

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A NEW HUMAN NEMATODE *STRONGYLUS GIBSONI*, n. sp.

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Some time ago I received from Dr J. Bell, Civil Hospital, Hong Kong, some nematodes for diagnosis. The following history accompanied them:—'They were found by Mr. Adam Gibson, Colonial Veterinary Surgeon, in the faeces of a Chinaman employed at the Slaughter-house, Hong Kong.'

There were ten ♂♂ and nine ♀♀. On examining the males, it was evident that they were probably new species of Strongylidae from the configuration of the male bursa and the extremely long delicate spicules which were extruded in several of the specimens. Prof. Looss, to whom I sent them for examination, wrote: 'The worms you sent are new to me; I only know one similar form which I found in hares from the neighbourhood of Sawakin.' The state of preservation was unfortunately very poor, the specimens being much distorted, and giving the impression of having been partially dried at some time or other. The bursae, however, had preserved their contour fairly well, and in one or two specimens it was possible to observe the uterine and anal openings in the female.

The male.—21 mm. long and 0·4 mm. thick towards middle. Head attenuated. Two lateral papillae occur, one on either side of the buccal orifice. About 0·45 mm. behind the head are situated two cervical papillae (fig. 1).

The Bursa.—Visible to the naked eye is a bilateral appendage. Each lobe is a concave-convex lanceolate expansion, the tip of one folding over that of the other. The plane of the lobes lies in the dorso-ventral plane. Dorsally the edges of the lobe are continued forwards, meeting to form a long V-shaped slit, while posteriorly the edges run a parallel course until they curve inwards at the tip (fig. 2). Ventrally the lobes have, a little in front of their origin, two well-

marked sub-conical lobules, otherwise the ventral edge is uniformly curved from base to apex (fig. 3).

The disposition of the rays as far as could be seen was as follows:

Dorsally. An (anterior) ray terminates in a papilla about the middle of the dorsal edge; (*a*) behind this are two (median) rays, the posterior of which is the larger, and is curved inwards before it reaches the margin. Finally two rays diverging from a common stem end in papillae at the tip of the bursa (fig. 4).

Ventrally. A ray terminates in each of the ventral lobules (fig. 5). It was not possible to trace any of these rays from their origin from the central mass.

The distance from the base of the bursa (dorsally) to the tip of the folded and curved lobes is about a millimetre.

Appendages of the genito-anal opening.—Dorsally there is a prolongation of the subcuticular layer, which bifurcates posteriorly into two appendages which appear to have papillae. Anteriorly this is bounded by a crescentic line (fig. 6). Laterally on either side there is a small lobule.

Ventrally there is a conical plate with chitinous margin, and closely applied to it dorsally a larger crescentic plate, also with chitinous margins, the anterior portion of which is continuous with the ventral edge of the bursa (fig. 7).

The Spicules are about 7 mm. long, thus occupying about one-third of the length of the body. In figs. 8 and 9 they are shown in different stages of extension.

The female is about 25 mm. long. The tail end is pointed, and in some specimens somewhat curved. The anus is situated 0·2 mm. and the uterine opening 0·5 mm. from the tip. The eggs in utero measure 110 × 53 μ (fig. 10).

The worms are represented in their natural size in fig. 11.


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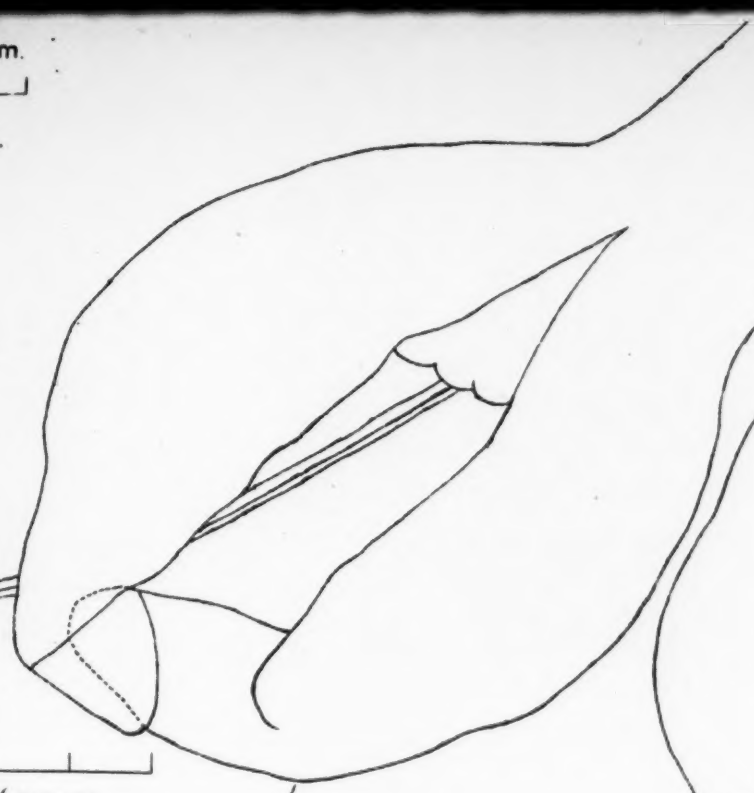
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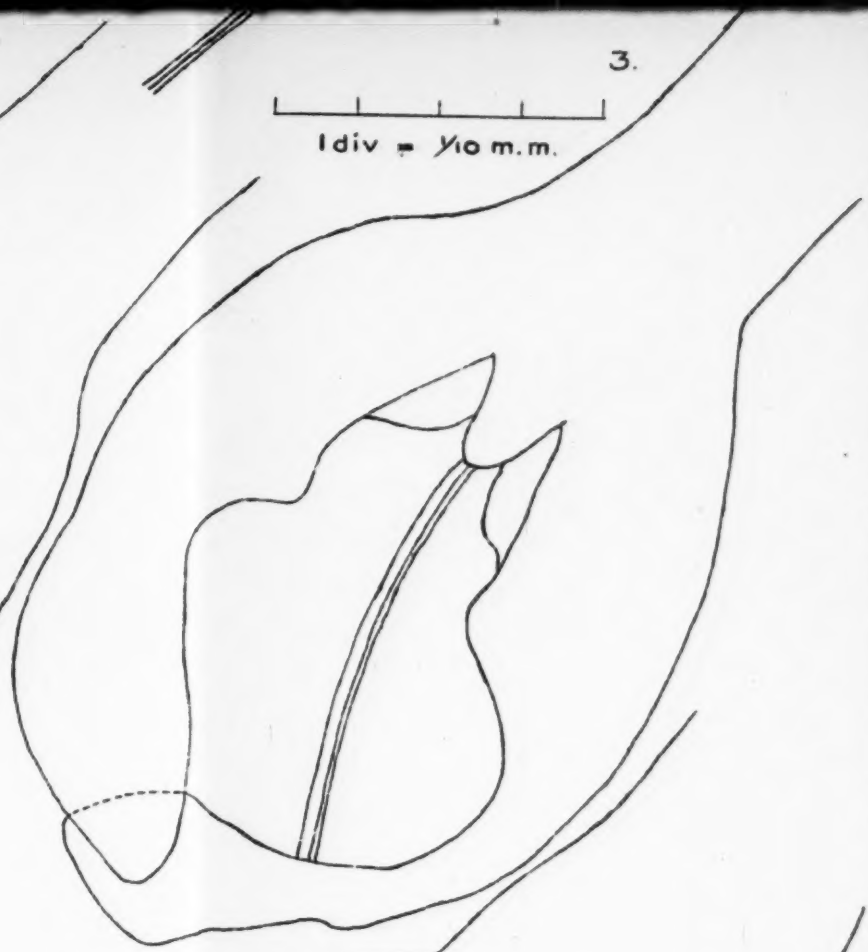
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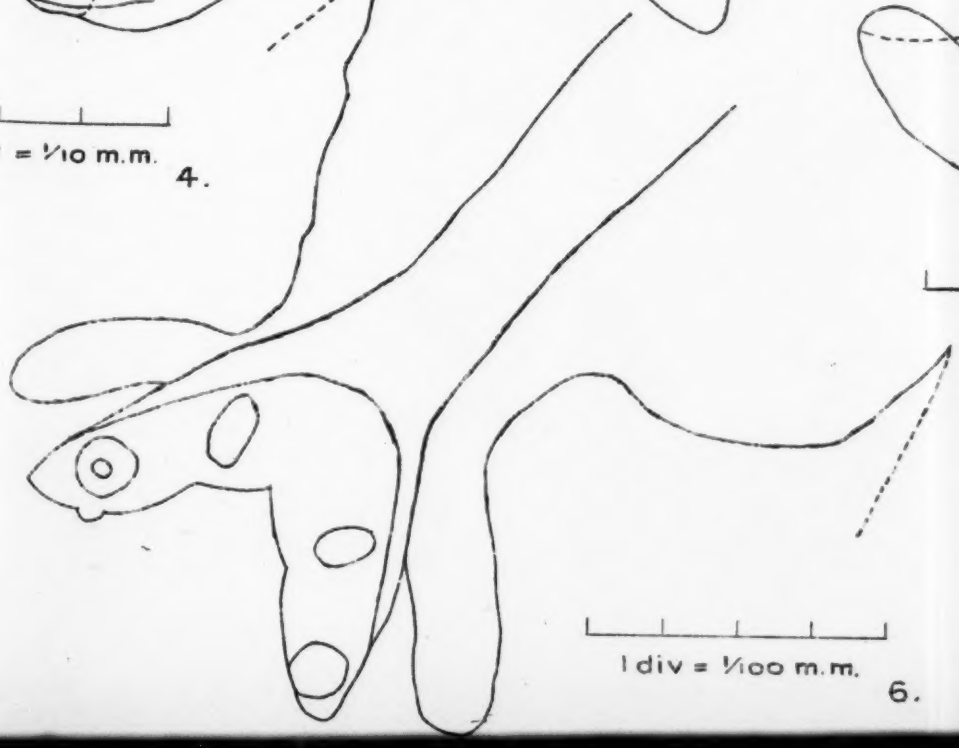
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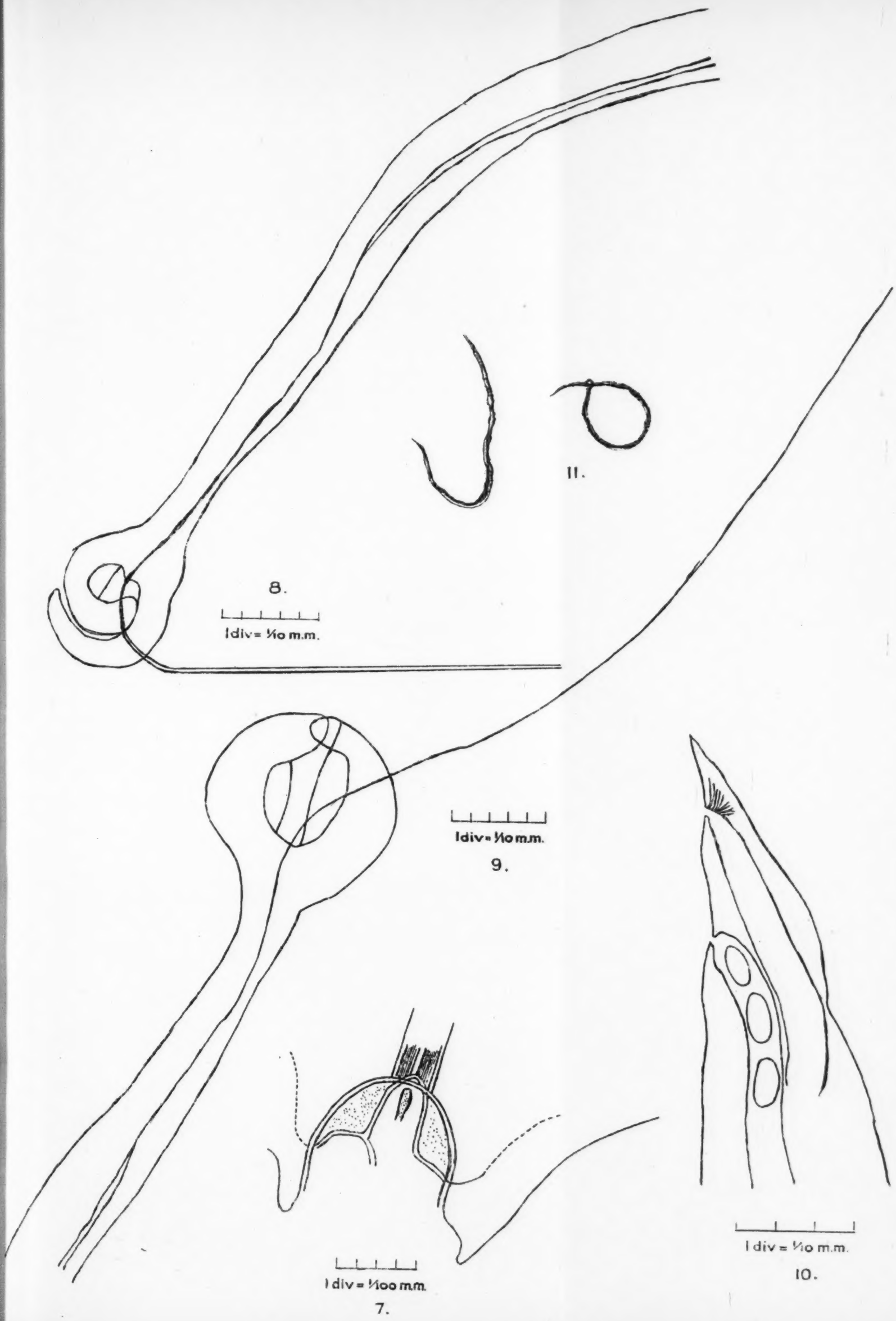
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ON THE SUPPOSED OCCURRENCE OF *FILARIA IMMITIS* IN MAN

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Over and over again the statement occurs in text books of medicine and parasitology that this worm has been found by Bowlby in man. Not infrequently doubt is thrown on the accuracy of the identification of the parasite by Bowlby. On referring recently to Bowlby's communication, I was surprised to find that the worms found by Bowlby were Bilharzia and that no mention whatever is made of *Filaria immitis*. Further I found that this fact had already been pointed out by Moniez* and an explanation given of how the error arose. As, however, this passage in Moniez appears to have been overlooked, I think it advisable to call attention once more to the facts.

The following is the report† of the communication made by Bowlby to the Pathological Society of London.

'Mr. Bowlby exhibited the urinary organs and parts of other viscera removed from two cases of Bilharzia. The first patient was an Arab, who was admitted into the hospital at Alexandria under the care of Dr. Mackie. The man was suffering from severe cystitis, with foul, blood-stained urine. On examination the bladder was found to be greatly thickened, and felt as though it contained a malignant tumour. In the urine the ova of the bilharzia worm were found. Perineal cystotomy was performed to relieve the patient's suffering, but he died a fortnight later. At the post-mortem examination numerous female bilharzia worms were found in the portal vein, thirty-seven of which, together with the urinary organs and portions of the lungs and spleen, had been sent by Dr. Mackie

* Traité de Parasitologie animale et végétale appliquée à la medicine, 1896, p. 356.

† Lancet, 20th April, 1889, p. 786.

to Mr. Bowlby. The bladder was contracted and thickened. The mucous coat was covered with a shreddy mass of finely fibrillated villous growth. The ureters were dilated, with thickened walls, and the mucous membrane was covered by a phosphatic deposit. The kidneys were in a state of suppuration. Under the microscope the thickening of the bladder wall was found to be due to an interstitial overgrowth of fibrous tissue, and the mucous membrane had been destroyed and was replaced by young fibrous tissue. There were numerous ova imbedded in the wall of the bladder. The walls of the ureters were filled with ova, some of which could be seen in the mucous membrane where it had not been destroyed. The kidneys showed the changes due to nephritis, and contained numerous ova. The lungs were semi-solid; several ova scattered through them. The second patient was a boy, seventeen years of age, from whose rectum Dr. Mackie removed a tumour (exhibited). The patient had suffered from rectal pain and the passage of blood. The tumour consisted of a diffuse papillomatous growth, which under the microscope was found to consist of a loose, richly cellular, fibrous tissue, in the interstices of which were numerous ova.

'Dr. Stephen Mackenzie said the opportunity of studying the general pathology of this disease was rare. He asked what was the origin of the coagula and fibrous threads often seen in the urine in these cases. Were any parent worms found in the bladder? They nearly always inhabited the blood-vessels. The ova in the alimentary canal were said to have lateral spines, while those in the urinary tract had terminal ones.—Dr. Moore had found both lateral and terminal spines on ova in both situations.—Mr. Bowlby, in reply, said that the process was composed of young fibrous tissue in a state of disintegration. The parasites were only found in the portal vein.'

Then, as already pointed out by Moniez, an abstract with the following title appeared in September, 1889, in the Centralblatt für Bakteriologie, Bd. VI, 1889, p. 190:—

'Bowlby Mittheilung über 2 Fälle von *Filaria immitis* beim Menschen (Lancet, Vol. I, No. 16, p. 786).

'(1) Bei der Sektion eines Arabers, welcher an Blutharnen gelitten hatte und dessen Blasenwand sich bei Lebzeiten schon verdickt anfühlte, fanden sich in der Vena portarum zahlreiche weibliche Würmer. In der stark verdickten Blasenwand waren

zahlreiche Eier eingebettet. Auch in den Harnleitern und Nieren fanden sie sich, sowie, in geringer Zahl, in den etwas derb anzufühlenden Lungen. (2) Bei einem 17 jahringen Knaben wurde ein Tumor im Rectum entfernt. Derselbe erwies sich als aus einem lockeren reichlich zellenhaltigen, fasrigen Gewebe bestehendm in dessen Zwischenräumen zahlreiche Eier lagen.' Kurth (Berlin).

This accidental or erroneous use of the name *Filaria immitis* for the worms, which as the original shows were Bilharzia, has probably been the source of all the following erroneous misquotations. At any rate it is clear that in these cases there is no question of *Filaria immitis* but of Bilharzia.

To make quite certain of this I wrote to Mr. Bowlby, and the following is an extract from his reply to my letter :—' I did not know before that I was supposed to be the discoverer of this Filaria. I am quite innocent of any knowledge of the said parasite, and the paper you refer to was on some cases of Bilharzia. If you can correct the error, please do.' This then disposes of Bowlby's supposed record.

Finally there is the record by Braun* of the supposed occurrence of *F. immitis* in man. Braun's words are the following :—' Ich fuhre hierbei dass im Jahre 1885, in Dorpat in der leiche eines Russen die zu Präparierübungen benutzt wurde sehr lange Nematoden in grosserer Zahl in den Venen gefunden worden sind ; ich habe die wohl-erhaltenen Würmer selbst gesehen und korserviert ; an ihren Filarien natur ist nicht zu zweifeln, jedoch bin ich nicht imstande mehre auszusagen, da ich die Parasiten nicht mehr untersuchen konnte.'

In this case, however, as the species was not identified, I do not think we are at present justified in including *F. immitis* among the parasites of man.

* Braun, Max. Die Tierischen Parasiten des Menschen, Vierte Auflage, p. 295.

A NEW POROCEPHALUS (*POROCEPHALUS CERCOPITHECI* n. sp.)

BY

ANTON BREINL AND EDWARD HINDLE

From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine

(Received for publication 9 December, 1908)

At the autopsy on one of our experimental monkeys (a large female *Cercopithecus callitrichus*), in the left lower lobe of the lung, a subpleural cyst about 3 mm. in diameter was noticed. This cyst contained an immature example of a Porocephalus coiled up inside it. Although the whole lung was carefully dissected, no other specimens were found; the other organs and the gut were also searched without success.

FIG. 1.

The body is a uniform greyish white in colour (fig. 1). It exhibits a separation into head (cephalo-thorax?) and body, the latter being divided into about 45 annuli, which become very indistinct towards the posterior end. The distance between the annuli is greatest towards the anterior end, and gradually diminishes posteriorly.

The dimensions of this specimen are:—Length, 10 mm.; diameter, at anterior extremity, 1·17 mm.; and diameter at posterior extremity, 0·90 mm.

The short, bluntly-rounded head (fig. 2) is cut off from the body by a distinct groove passing completely round the animal. The dorsal surface of the head is slightly curved. The ventral surface is convex and bears two pairs of hooks, one on each side of the median mouth. This latter is surrounded by a chitinous ring, oval in shape, about 0·19 mm. in length, and 0·8 mm. in breadth. The inner hooks are single, each consisting of a stout, strongly-curved chitinous process 0·24 mm. in length. In the outer pair, from the base of each hook arises a slightly curved unciform appendage (0·11 mm. in length), as in all the hooks of *Porocephalus najae-sputatricis*, Leuckart. The hooks of both the outer and inner parts are jointed at their bases.

The body exhibits a median line running along the ventral surface to the posterior extremity.

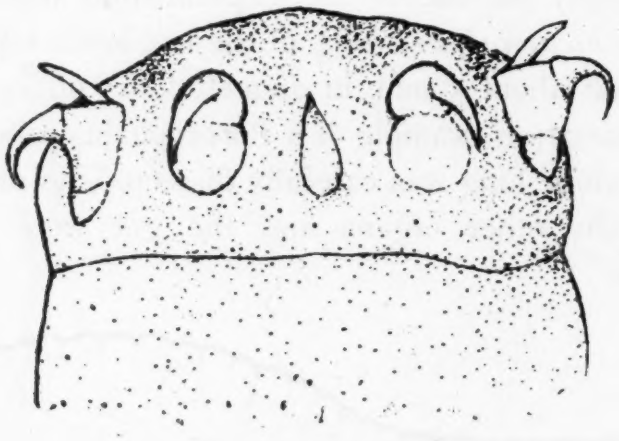


FIG. 2.

The anus is a small aperture situated at the apex of a small papilla at the posterior extremity. Immediately beneath it is the genital aperture.

This species is distinguished from the known species of Porocephalus by the presence of an appendage on the *outer* pair of hooks only. We propose the name *Porocephalus cercopitheci* in view of the host.

The type specimen of this species is in the museum of the Liverpool School of Tropical Medicine.

COMPARATIVE CHEMO-THERAPEUTICAL STUDY OF ATOXYL AND TRYPANOCIDES

PART II

BY

M. NIERENSTEIN, PH.D.,

J. W. GARRETT INTERNATIONAL FELLOW AND RESEARCH DEMONSTRATOR, LIVERPOOL SCHOOL OF TROPICAL MEDICINE

From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine

(Received for publication 9 *December*, 1908*)*

Our previous work[1] on the chemo-therapeutics of Atoxyl has led us to the conclusion that a combination *in vitro* takes place between proteins, and Atoxyl mono-acetylated Atoxyl and mono-benzoylated Atoxyl respectively; whilst, on the other hand, such a combination does not occur between proteins, and sodium arsenate acetyl-benzoyl Atoxyl and sodium-p-hydroxy-phenyl-arsenate. This work has been continued by injecting the above-mentioned drugs into experimental animals; these reacting in an analogous way to the serum-proteins *in vitro*, with only one exception—acetyl-benzoyl Atoxyl—which combined with the serum proteins *in vivo*. This reaction, however, was only to be expected, as the organism saponifies the acetyl group, and the resulting benzoyl Atoxyl acts in the same way as mono-benzoyl Atoxyl *in vitro*.

Technique.—Rabbits were injected for several months, twice weekly, with Atoxyl, Sodium arsenate, acetylated and benzoylated Atoxyl, Benzoyl-acetyl Atoxyl, and Sodium-hydroxyl-phenyl-arsenate. Usually 1 c.c. of 1 per cent. solution of the drug was injected.

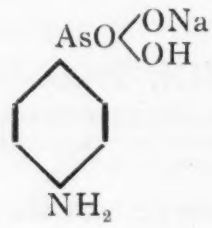
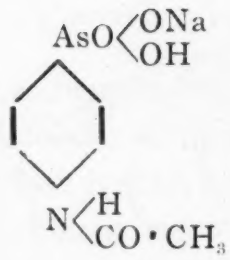
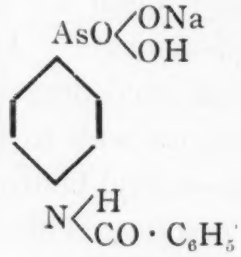
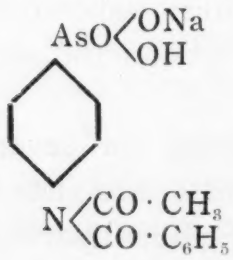
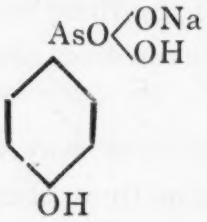
After a time, 20 c.c. of blood was taken from the jugular vein, and the serum used for analysis.

The arsenic was estimated in the same way as in our previous work; the slightly modified Sanger's method being adopted. Gold chloride was used as developer in preference to hydrochloric acid.

The results of the experiments are given in the following table

(Table I). For comparison, our previously recorded observations *in vitro* are appended:—

TABLE I

Drug	Chemical Constitution	Arsenic Estimation *in vivo*	Arsenic Estimation *in vitro*	Number of Experiments *in vivo*
Atoxyl		arsenic present	arsenic present	5
Sodium arsenate	—	arsenic* absent	arsenic absent	5
Acetylated Atoxyl		arsenic present	arsenic present	2
Benzoylated Atoxyl		arsenic present	arsenic present	2
Benzoyl-acetyl Atoxyl		arsenic present	arsenic absent	2
Sodium-p-hydroxyl-phenyl-arsenate		arsenic absent	arsenic absent	2

* In one of these experiments arsenic was found to be present. The fact that this particular serum contained haemoglobin may explain the exception.

Similar experiments were carried out on two donkeys in order to obtain larger quantities of blood, so as to make a more detailed examination of the distribution of the arsenic with regard to the constituents of the blood.

This table shows that the haemoglobin contained arsenic in both cases :—

TABLE II

	Atoxyl	Sodium Arsenate
Haemoglobin	arsenic present	arsenic present
Stroma	arsenic present	arsenic absent
Serum	arsenic present	arsenic absent

The above recorded experiments confirm and extend the view that the amido group in Atoxyl and allied compounds *in vitro*, as well as *in vivo*, combines with the serum proteins.

After the mode of the combination of Atoxyl and serum proteins in the animal organism had been established, it seemed necessary to estimate the amount of Atoxyl which is secreted in order to form an idea as to how much of the drug is actually left in the body.

For this purpose a horse was injected subcutaneously with Atoxyl, and the urine and faeces analysed. The arsenic was estimated according to Dupas-Gilier's[2] iodine method, using Gileas's and Shearer's modification.

Table III gives the amount of Atoxyl injected and recovered in urine and faeces :—

TABLE III

Urine

Date of Injection	Amount Injected	Date of Collecting	Arsenic Recovere
28.2.08	1 gm. Atoxyl	1.3.08	81%
2.3.08	1 gm. Atoxyl	3.3.08	83%
5.3.08	1 gm. Atoxyl	6.3.08	78%
7.3.08	1 gm. Atoxyl	8.3.08	82%
10.3.08	2 gm. Atoxyl	11.3.08	79%
12.3.08	2 gm. Atoxyl	13.3.08	85%
16.3.08	2 gm. Atoxyl	17.3.08	82%
19.3.08	2 gm. Atoxyl	20.3.08	80%
22.3.08	1 gm. Atoxyl	23.3.08	86%

Faeces

Date of Injection	Amount Injected	Date of Collecting	Arsenic Recovered
12.3.08	2 gm. Atoxyl	13.3.08	4%
16.3.08	2 gm. Atoxyl	17.3.08	2%
19.3.08	2 gm. Atoxyl	20.3.08	5%

The chemical details of this work will be published shortly.

Atoxyl has been found to be secreted in the urine, as :—

(1) p-amino-phenyl-arsenious acid;
(2) p-oxy-phenyl-arsenious acid;
(3) Arsyl-oxy-carbonyl.

The presence of this third compound in the urine is of practical interest, in so far as its formation can only be explained by assuming that Atoxyl is acetylated in the organism, and afterwards the p-amino-phenyl-arsenious acid is transformed into Arsyl-oxy-carbonyl. Similar observations[3] have been made on the secretion of p-toluidine, in which case Methyl-oxy-carbonyl is formed. It is quite possible that this may explain the fact that acetylated Atoxyl is less toxic than Atoxyl.

Besides arsenic, anilin was found to be present, *only* in the faeces. The faeces were treated with alkali, and steam passed through the mixture. The condensed steam gave distinctly, with bleaching powder, Hoffmann's reaction.

With regard to the distribution of Atoxyl in the organism, it has been shown by different authors that arsenic is deposited in all organs to a greater or lesser extent. J. Magalháes[4] states that Atoxyl does not permeate the meninges. We have attempted to verify his statement by using the cerebro-spinal fluid of infected donkeys withdrawn at different intervals after the last administration of the drug.

Table IV gives the results of the analysis :—

TABLE IV

Days elapsed since last Injection	Arsenic Examination
7 days	arsenic present
1 day	arsenic absent
9 days	arsenic absent
2 days	arsenic present
5 days	arsenic present
9 days	arsenic absent
3 days	arsenic present
6 days	arsenic present

These experiments prove conclusively that the meninges are permeable to Atoxyl, as in the majority of the recorded cases arsenic could be detected in the cerebro-spinal fluid.

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2. Zeitschrift für angewandte Chemie, Vol. 22, p. 378.
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4. JOSÉ DE MAGALHÁES. Etude au point de vue therapeutique de la perméabilite meningée dans la Trypanosomiase humaine. XV Congrès International de Medicine, Lisbonne, 1906. Fascicule 2, p. 304 ; see also BREINL and TODD, British Medical Journal, 1907, January 19th, p. 132.

CHEMICAL NOTES ON ATOXYL

BY
M. NIERENSTEIN, PH.D.

From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine

(Received for publication 9 December, 1908)

The following is a record of chemical observations made during the last two and a half years whilst working on the chemical constitution and physiological action of Atoxyl.

Water of crystallisation.—The differences in the amount of arsenic found in samples of Atoxyl due to the differences in the water of crystallisation (Moore, Nierenstein and Todd,[1] Ehrlich and Bertheim,[2] and others), suggested an exact estimation of the water of crystallisation and 'adhering moisture.' Experiments have proved that Atoxyl contains from one half to one and a half molecules of adhering moisture, and exactly two molecules of water of crystallisation. Further observations showed that Atoxyl loses the adhering moisture in about *five weeks* when standing over concentrated sulphuric acid; after this time no further loss of weight could be noted in the course of six months. It contains, then, two molecules of water of crystallisation which are only lost on drying for three hours at 160° C. Atoxyl, after having been kept over sulphuric acid for at least five weeks, has, therefore, the formula:—

$$H_2N \cdot C_6H_4 \cdot AsO\left\langle\begin{matrix}OH\\ONa\end{matrix}\right. + 2\,H_2O, \text{ corresponding to } 23{\cdot}88\% \text{ arsenic.}$$

Therefore, in order to administer always the same amount of arsenic in a given dose of Atoxyl, it is advisable that Atoxyl should be kept in a dark desiccator over concentrated sulphuric acid for at least five weeks before use. The dark desiccator ought to be used on account of the decomposition of Atoxyl solutions when exposed to light for some time.

Inorganic arsenic.—Two distinct brands of Atoxyl manufactured by the Vereinigte Chemische Werke, Charlottenberg, have been

supplied to us since 1905; one having the appearance of a white powder, the other of distinct crystals. It was found that the powder contained free inorganic arsenic (from 0·4 to 0·9%), which is easily detected by passing H_2S into the *slightly* acidified solution. The crystalline Atoxyl did not contain any inorganic arsenic.

Parafuchsin.—On two occasions it was noticed that the freshly prepared solution of Atoxyl was of a red colour; this was due to the presence of parafuchsin, which is formed as a by-product during the preparation of the drug.

Yellow Atoxyl.—One special supply of Atoxyl sent out to Uganda gave rise, in the hands of Captain A. C. H. Gray,[3] on injection, to violent toxic symptoms and blindness. The qualitative examination of the drug showed that it dissolved in strong alkali (40% KOH) with a yellow colour; this colour reaction has never been observed by us in any other sample of Atoxyl. This Atoxyl contained free inorganic arsenic, traces of free anilin, and a second substance which is probably an oxidation product of Atoxyl. It is quite possible that this substance may be responsible for the untoward effects.

Therefore, it would seem advisable that Atoxyl should be tested with strong alkali before use. 1 c.c. of a 5% solution should be mixed with 2 c.c. of strong alkali and left standing for a few minutes, and if the solution shows a yellow coloration the Atoxyl should not be used for treatment.

A full chemical report on the 'yellow Atoxyl' has previously been published.[3]

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1. B. Moore, Nierenstein and Todd. Bio-chemical Journal, Vol. II, 1907, p. 300.
2. Ehrlich and Bertheim. Berichte d. deutschen chemischen Gesellschaft, Vol. 40, p. 3296.
3. A. C. H. Gray. Quarterly Report on the Progress of Sleeping Sickness and Medical Treatment of Sleeping Sickness in Uganda, 1908, pp. 32 and 35.

NOTE SUR LE RÔLE DES TABANIDES DANS LA PROPAGATION DES TRYPANOSOMIASES

PAR

LE DR. EDMOND SERGENT

(Received for publication 25 January, 1909)

Récemment plusieurs auteurs anglais ont bien voulu rapeller les expériences que nous avons faites sur la transmission des trypanosomiases par les insectes piqueurs. Mais leurs citations contiennent parfois une erreur, involontaire, et qui consiste à nous faire dire que nous avons réussi à donner le *nagana* avec des Taons qui avaient piqué 48 heures auparavant un animal infecté.* L'erreur provient de ce que ces auteurs ont dû se documenter sur le tableau page 679 du tome XX *des Annales de l'Institut Pasteur*, tableau dans lequel s'est glissée une erreur de typographie faisant attribuer au nagana ce qui revient au *debab*. Cette erreur a été corrigée dans un erratum publié page 880 du même tome XX *des Annales de l'Institut Pasteur*, erratum qui a évidemment échappé aux auteurs en question.

Comme cette question de la transmission des trypanosomiases est à l'ordre du jour je crois utile de rappeler ce qu'ont été nos expériences relatives au debab, que l'on trouvera *in-extenso* dans les *Annales de l'Institut Pasteur* du 20 janvier 1905 (T. XIX), pages 31 à 41.

Nous avons montré que les deux espèces de Taons le plus repandues dans le Tell algérien: *Atylotus tomentosus* et *Atylotus nemoralis* peuvent transmettre expérimentalement l'infection d'un animal malade à un animal sain quand les piqûres le suivent immédiatement. Dans une expérience nous avons établi que la piqûre de Taons ayant sucé le sang d'un animal malade peut infecter un animal sain encore après 22 heures d'intervalle entre les deux piqûres.

* Dutton, Todd et Hanington. 'Annals of Trop. Med. and Parasit.,' Vol. I, 2nd note, p. 212.

Kinghorn et Montgomery. 'Annals of Trop. Med. and Parasit.,' Vol. II, p. 86.

supplied to us since 1905; one having the appearance of a white powder, the other of distinct crystals. It was found that the powder contained free inorganic arsenic (from 0·4 to 0·9%), which is easily detected by passing H_2S into the *slightly* acidified solution. The crystalline Atoxyl did not contain any inorganic arsenic.

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ON THE NOMENCLATURE OF THE MAMMALIAN TRYPANOSOMES OBSERVED IN NORTH WESTERN RHODESIA

BY

R. EUSTACE MONTGOMERY, M.R.C.V.S.,

AND

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Of the Expedition of the Liverpool School of Tropical Medicine to the Zambesi, 1907-1909

(Received for publication 15 *December*, 1908*)*

In view of the conclusions at which we arrived as to the nature of the trypanosomes found in bovines of North Western Rhodesia, it appears desirable to briefly outline the subject from the standpoint we have taken.[1] We would observe that at the time our report was despatched from the Luapula we had before us, with small exception, only literature bearing date prior to April, 1907, and on the evidence therein contained, we did not feel justified in discussing the question of the morphology or specificity of our trypanosomes more fully than was done, and it will no doubt have seemed to one noting only the date of publication as though our duty had been somewhat neglected, and our conclusions based in defiance of or without due regard to the results of those workers whose communications had been public property in Europe for upwards of fifteen months prior to the appearance of our report. Had we before us at the present moment only the evidence already advanced, we would hesitate to re-open the question; but the perusal of the literature now with us, and more especially our own more recent observations on trypanosomiasis in other areas of Northern Rhodesia, justify, we consider, this further exposition. In a forthcoming report we shall have occasion to refer to and to compare with that on trypanosomiasis in North Western Rhodesia; and since we purpose utilising this as a basis for critical comparison, it is highly desirable that our position and views

concerning these trypanosomes, to which we have referred as *T. dimorphon* and *T. vivax*, should be most clearly stated; so that, whatever be the faults in our deductions, these may be recognised and corrected by those to whom the necessary facilities are available. Owing to our protracted absence from Europe and the relative paucity and delay in the receipt of current scientific literature, we shall be precise in specifying all communications referred to, since it is not unlikely that at the time of writing important papers bearing on this particular case may have appeared in print, but will remain inaccessible to us for some months.

Probably no sub-division of the Protozoa presents more difficulties in the way of classification than does the genus *Trypanosoma* Gruby, emended by Laveran and Mesnil in 1901 so as to exclude those organisms of fish which carry both an anterior and a posterior flagellum. On morphological grounds Lühe has created the genus *Trypanozoon* to contain only those trypanosomes parasitic in the blood of Mammals; beyond this no further attempt has been made to classify or arrange the genus; and, indeed, any additional sub-division would be impracticable to a Zoologist. We are in a transition stage, when rumours of multiplication cycles, resting stages and forms which from their appearance are unrecognisable, even as flagellates, are abroad. At the present time, we have positive knowledge only of the well-known forms found free-swimming in the blood; and these presenting such close analogies to one another, offer no opportunity to the Protozoologist of satisfactorily adopting any further classification based on morphological features. It is possible that the more exact and more strictly cytological technique which is now being employed in the study of these organisms may bring to light some new features in their structure to serve as a fixed point from which morphological classification can commence; but until it has been employed uniformly on all known species, and critical comparisons made, it is of little more value than the older methods used both by Zoologists and Pathologists.

Any classification dependent upon a variable feature and allowing margins to the personal equation must be prone to error, and cannot be unquestionably accepted. Yet we find that Zoologists and Pathologists alike accept as species forms of trypanosomes distinguished from others on morphological grounds. *T. dimorphon*

is admitted to be a 'good species,' and solely from its morphology; *T. equinum* and *T. theileri*, the former more especially, are also constituted largely from their unique appearance in the blood.

A clinician may subdivide the diseases due to trypanosomes on symptomatological or epidemiological grounds: and a pathologist may add to these the results of his observations on animals experimentally infected. Here again absolute distinction between any two species may be impossible, even though it be known that the morphology of each is markedly different. Thus the experimental disease, or the 'animal reactions' due to *T. evansi* and *T. dimorphon*, show very little variation in most Mammals; yet these organisms are clearly distinguishable on morphological grounds. Again, it would be a matter of the utmost difficulty to state specifically that *T. gambiense* was not the same as *T. evansi*, if only dead organisms were available. But if experimental animal reactions are considered, and the natural disease due to each be observed, they can be most strikingly separated.

However lacking in conformity to Zoological rules, and however imperfect and crude, it is possible, by adopting a combination of the results given us by morphological and pathological studies, to constitute various groups of mammalian trypanosomes, which to a worker removed from such facilities as exist in the research centres of Europe are, to our mind, helpful. An observer in the field may place a detected trypanosome, tentatively, in one of the classes with a minimum amount of work, and since he will be in possession of only a modicum of research equipment and a limited stock of experimental animals, he will be in a position to utilise these to their best advantage, and by adopting a certain degree of uniformity can bring his observations into line with all work previously carried out on the class of organisms to which his own approximates. Absolute differentiation of closely related trypanosomes is quite impossible to such a worker: a rigorous and critical morphological comparison must be effected, and final diagnosis will rest upon the result of this and that of 'cross-inoculation' into animals believed to be immunised against the organisms with which comparison is being made.

For such work 'types' of known and approved origin must be at hand, as well as the necessary animals for cross-inoculation. This must remain the work of an acknowledged centre, assuming the

functions of a museum wherein all type species are maintained and strictly guarded.

If we eliminate *T. lewisi* and allied species as not occurring in the blood of the higher mammals, there remain thirteen named species all more or less accepted and distinctive, which an observer may meet in man or the domestic animals. These are *T. gambiense*, *T. evansi*, *T. brucei*, *T. equiperdum*, *T. equinum*, *T. dimorphon*, *T. theileri* (including some of Lingard's forms met in India), *T. vivax*, *T. nanum*, *T. congolense*, *T. cazalboui*, *T. pecaudi*, and *T. sudanense*. On morphological grounds it is possible to distinguish:—

T. theileri, on account of its relatively immense size, and further from its animal reactions, being parasitic only in the blood of bovines. Dutton and Todd, however, describe a trypanosome morphologically recalling this organism as occurring in the blood of a *Tragelaphus scriptus* in the Congo Free State.[2]

T. equinum, which in stained preparation (dry method) shows a uniquely small blepharoplast. On epidemiological grounds in addition, by being limited to South America.

By adopting certain standards of animal reaction, we can regard as distinctive:—

T. gambiense, as being the only one, so far as known, pathogenic to man. The human subject not being available for inoculation, the diagnosis of forms recalling this species morphologically would remain work for comparison with types.

T. equiperdum, on the grounds of production in equines of quite characteristic clinical symptoms; and being unique among trypanosomiases of the lower animals in transference naturally by coitus.

Nine named species remain to be dealt with. By the adoption of morphological and animal reaction standards these can be subdivided into three groups, having as their types, in point of view of priority, *T. evansi*, *T. dimorphon*, and *T. nanum*, respectively.

The standards we adopt are arbitrary, and are open to criticism, as any must be which depend upon more or less relative features of size and pathogenicity.

Considerable variations are noticeable in the appearance of individual trypanosomes of any strain: we have the so-called 'male' and 'female' elements; and gross measurements are found to vary. But, be it noted, these variations are within limits. It would be most exceptional to find, for instance, any *T. evansi* of less than 20μ or of more than 35μ in length; and despite the tens of generations which many trypanosome strains have produced in various laboratories, we find the morphological features remain practically constant. There is not that tendency to develop new types, to revert to an old form, or otherwise to vary, which has more than once been suggested. We may with comparative safety, then, group into one class some of those nine species which conform approximately to the dimensions of *T. evansi.* Disregarding the differences in 'male, 'female' and 'indifferent' forms, these species would be monomorphic and of relatively large size.

On the other hand, there is a group of three species the morphological appearances of which differ markedly. In the course of infection by *T. dimorphon*, forms closely resembling *T. evansi* are encountered, but at the same time, or in the same animal, or, capable of production in another animal, trypanosomes markedly smaller, measuring only 10μ to 15μ in length, and without any free flagellum, make their appearance. It has been shown that these are but various manifestations of the same organism, which is consequently far from being monomorphic.

Of the nine species to which we refer, six show similar reactions towards experimental laboratory animals; three differ markedly. The value of animal reactions may be disputed, and when limited in amount, results are apt to cause confusion. Particularly is this the case with the donkey, cattle, sheep and goats, largely, we think, owing to the multitude of races and breeds employed, and also because many of them are country-bred animals whose ancestors have probably been exposed for generations to trypanosome infections. These animals, too, show a degree of individual idiosyncrasy which is prone to give rise to error. Monkeys, especially *Cercopithecus* and *rhesus*, domestic rabbits, guinea-pigs, white rats and tame mice, and to a slightly less degree, dogs of both European and native blood, are, however, fairly constant in their susceptibility or their insusceptibility to any one strain of trypanosome. During the many generations

which have been maintained of such organisms as *T. gambiense*, *T. evansi* and *T. dimorphon* in all the usual laboratory animals, there has never been any suggestion that a new type has been created. Increased and decreased virulence may and does occur, but this is less marked, under normal maintenance of the strain, than was hitherto supposed, and there is always a tendency to reversion towards the original state.

By means of these two factors the nine remaining trypanosomes can be conveniently grouped as follows:—

A. Trypanosomes, pathogenic towards most domestic animals, and producing a rapidly fatal infection in the usual laboratory animals:

(*a*) monomorphic, of large and fairly constant size, from 20μ to 35μ in length, and carrying a distinct free flagellum. Type—*T. evansi.*

(*b*) di- or polymorphic, of very variable size, occurring in at least two forms: (1) 'short,' measuring from 10μ to 15μ in length, and devoid of a free flagellum; (2) 'long,' which may attain 35μ, and bearing a variable length of flagellum. Intermediate forms also occur. Type—*T. dimorphon.*

B. Trypanosomes, pathogenic to certain domestic animals, and without apparent effect when inoculated into the usual laboratory animals (monkey, dog, rabbit, guinea-pig, rat and mouse). An imperfectly studied group, which includes *T. nanum*, *T. vivax* and *T. cazalboui.*

We consider this grouping to be sufficiently exact for the temporary purposes of a worker in the Tropics. Unless the organism with which he is dealing shows any striking peculiarity in either morphology or animal reaction, he can do little more than assign it to one of these groups.

In North Western Rhodesia we isolated from cattle, sheep and dogs three trypanosomes, one approximating to each of the groups, and we referred to these as *T. brucei*, *T. dimorphon* and *T. vivax* for the reasons set out below.

1. *T. evansi* group, which includes *T. brucei* and *T. sudanense*.[3]

The validity of *T. brucei*, save on the grounds of cross-inoculation, is open to question, unless it be pre-surmised that each species of trypanosome has its own particular genus or species of biting fly to bring about dissemination. In Africa, where both *T. brucei* and *T. evansi* occur, it has become usual to refer to *Glossina* and *Tabanidae* as the respective transmitters. In the same manner an *evansi*-like organism, which in nature is supposedly spread by *Glossina*, or which is met with in cases of 'tsetse-fly disease,' is referred to as *Trypanosoma brucei*. This, we take it, implies the acceptance of *T. brucei* as the local type of the *evansi* group, and we consequently assigned to it our dog trypanosome. But it is no more possible to distinguish between *T. evansi* and *T. brucei* in the field, unless the supposed transmitting factor be considered, than it is to differentiate *T. evansi* and *T. sudanense*.

2. *T. dimorphon* group, including *T. congolense*[4] and *T. pecaudi*.[3]

In their action on laboratory animals these three species coincide very closely; and in their morphology, as shown in stained film (dry method), *T. congolense* is almost identical with the *T. dimorphon* at Paris. Writing on the subject of these two, Martin, Leboeuf and Roubaud[5] hold that in the French Congo the latter is more active, moving more readily across the field and producing more extensive lateral displacement of the corpuscles; and they contend that a little experience will enable an observer to distinguish between the two. Assuming that these two species are distinct, based on the strains in Europe, it is not to be forgotten that in countries such as parts of Africa, where mixed infections are not unknown, they might conceivably both occur in the same host, and here more especially since the geographical distributions coincide.

T. pecaudi appears to have more claim to recognition in the field. Morphologically, the similarity between it and *T. dimorphon* is great, although it has been noted that the 'short' form of *T. pecaudi* may attain a greater breadth and carry a slightly better developed undulating membrane than is usual in the type strain, and both forms are present at the same time. Laveran,[3] however, states, 'these morphological differences do not suffice to differentiate the two

parasites.' It will be remembered that Dutton and Todd[6] were unable to adduce much information regarding the animal reactions in the course of the natural disease of the Gambia, except in horses. These correspond to what are manifested in 'baleri' as outlined by Cazalbou.[7] In experimental animals the results coincide: the disease is acutely fatal in rats, guinea-pigs and dogs, though Laveran has noticed some slight variations in the mouse, and in all animals, as in *dimorphon* and *congolense* infections, splenic enlargement is common. One further point of difference might be cited; in sheep and goats experimentally infected with *T. pecaudi*, the blood, though virulent on subinoculation, very rarely shows parasites; in *T. dimorphon* infection this is not usual. We have already referred to the variability of these animals under experimentation.

The parasite which was obtained in the majority of cattle at Broken Hill corresponded to the original description given by Dutton and Todd of *T. dimorphon*, in so far that all three forms, including that with a free flagellum, were found, and the animal reactions were similar. Since the free flagellated and the small forms were not present simultaneously, it is improbable that we were dealing with *T. pecaudi*; and as *T. congolense* does not appear to occur in a 'long' form it may also be negatived.

3. Group including *T. nanum*, *T. vivax* and *T. cazalboui*.

This group, constituted on the grounds of immunity enjoyed by the usual laboratory animals, presents difficulties in the way of subdivision, as from the very nature of the parasites they are more difficult of use in experimental observation, and hence less studied.

Of the three species, *T. nanum* takes priority; but, if we may judge from the very limited amount of work that has been possible, it is clearly separable from the other two on account of its morphological features. Laveran[8] describes it as only measuring 10μ to 14μ in length, and this in conjunction with Balfour's observations[9] that two monkeys, two rabbits and a dog were not infected are held to substantiate the species. It is true that the morphological appearances strikingly recall the 'tadpole' form of *T. dimorphon*; but though the number of inoculated animals be small, infection, in some at least, should have resulted had this organism, to which laboratory animals are highly susceptible, been employed. Further

work may regain *T. nanum* in the Sudan, when its position may be made more clear; until that time its specificity must remain questionable, though we incline to consider it as distinct from *T. dimorphon*,, and, therefore, to have no relation to the trypanosome of North Western Rhodesia which we have associated with that species.* Further, its small dimensions preclude the possibility of its connection with either *T. vivax* or *T. cazalboui.*

In July, 1906, Laveran[10] announced his belief that the trypanosome of 'La Soumaya' was a new species, to which he gave the name *T. cazalboui.* Up to this time the impression had been created by various writers that the causal agent of La Soumaya was *T. evansi*, and, indeed, such statements had been made. Laveran and Mesnil[11] say 'ils ont la plus grande resemblance avec le trypanosome de la Mbori,' and they proceed to quote Cazalbou's animal inoculations which resulted in the death of grey rats, mice and Sudanese dogs. In a later paper, published in May, 1907, Laveran[3] advances such additional evidence as to make the species incontestible. By morphology, animal reactions and cross-inoculations, *T. cazalboui* is clearly separated from the *evansi* and *dimorphon* groups, and from *T. nanum*, to which it is only related by the similarity of animal reactions. On all grounds it is a 'good species,' and one which can be detected and classified in the field with comparatively little trouble.

The same can hardly be said of *T. vivax*, as we yet know it, an organism described by Ziemann in 1905, a year before *T. cazalboui* was created, and given a specific name mainly on account of its rapidity of motion in cover-glass preparations. The morphology of this organism is in dispute: Ziemann[12] distinguishes it from *T. brucei*, though Laveran[13] was unable to note any difference from *T. evansi* in the film he examined; and Schilling[14] contends that it is but a slightly more rapid form of *T. brucei.* This opinion is not reflected by Lühe, who writes[15] 'Ich selbst finde in einem mir übersandten 'Originalpräparate die unter sich durchaus gleich gestalteten 'Trypanosomen kleiner wie *Tryp. brucei* . . . . mit nur schwach 'ausgebildeter undulierender Membran, wenig hervortretenden

* Wenyon (Report of the Wellcome Research Labs., Vol. 3) has apparently recovered *T. nanum.* We consider that one of the trypanosomes we have isolated from cattle, sheep and goats in North Eastern Rhodesia is closely allied to this species. (April 15th, 1909.)

'Bandsaum derselben sehr kurzer oder völlig fehlender freier Geissel, 'rundem Blepharoblast und nicht auffällig zugespitztem Hinterende.' 'Die Länge beträgt nach Ziemann 18-26, bisweilen bis zu 30μ, die 'Breite 2-2$\frac{1}{2}\mu$; der runde Blepharoblast liegt nahe dem meist etwas 'zugespitzten Hinterende.' To this we may add that Ziemann found difficulty in differentiating 'sexual' forms; an implication that this trypanosome is not subject to morphological variations of more than slight degree. Writing on *Trypanosoma cazalboui*, Laveran says 'its length, including flagellum, is 21μ, breadth 1·5μ. 'Nucleus oval and situated towards the middle. The centrosome 'round and distinct is placed near the posterior extremity, which is 'rounded and not pointed. Undulating membrane is very slightly 'developed, being little folded as in *T. lewisi*.'

A comparison of Lühe's and Laveran's descriptions of *T. vivax* and *T. cazalboui*, respectively, shows how little morphological difference there is between the organisms in stained film; and the similarity is accentuated when, referring to the movement in fresh preparation, Ziemann's organism, named on account of its motility, is described as moving '"wie einen Hecht" in mehr oder weniger 'gerader Linie quer durch das Gesichtsfeld schiessen lässt;' while *T. cazalboui* is said to be 'very active, moving sometimes on itself, 'at others soon leaving the field like an arrow.'

The animal reactions of *T. vivax* have been imperfectly studied. Comparing the natural disease induced in cattle, sheep and goats, we can note no great difference from La Soumaya. Experimentally, Ziemann is quoted by Sander and Hennig[16] as having had the following results: 'Graue Ratten, Tod nach 8-11 Tagen; Deutscher 'Hund (?); einheimischer Schweine: nur leichte Erkrankung; 'Esel: chronischer Verlauf . . . Ohne Erfolg: Katzen, Haus-'geflugeln, eine weisse Ratte.' Nabarro in his analysis of Ziemann's paper writes[17]: 'Dogs, cats and pigs were found not to suffer from 'the natural infection.' 'A dog and a native sucking-pig developed 'a slight temporary infection. White rats, geese, ducks, native hens, 'young turkeys, a native cat and an old pig were all refractory.' Despite the limitations of Ziemann's opportunities, it remains unquestionable that he was dealing with an organism showing marked lack of pathogenicity towards experimental animals. Laveran has not recorded the results following inoculation in grey rats, but

Cazalbou had previously noted their susceptibility to the trypanosome of La Soumaya.

It is most certainly to be regretted that *T. vivax* has not been placed on a more substantial footing; but the evidence that is before us indicates strongly that it has few if any affinities with either the *evansi* or *dimorphon* group, and we are unable to see wherein *T. cazalboui* differs in any manner from what has been made known regarding *T. vivax*, and as further indicating the resemblance between these two, it may be added that Tabanidae are blamed by the German writer for transmitting his species in the Cameroons, and Cazalbou on more than one occasion lately has emphasised his belief that this family is concerned in the dissemination of La Soumaya in the Niger regions.

Though they have never received specific designations, two other trypanosomiases of cattle deserve mention in connection with this group: that of Bruce, Nabarro and Greig at Entebbe,[18] and that described from Erythrea by Memmo, Martoglio and Adani.[19] In each instance the respective observers have noted the insusceptibility of laboratory animals to infection. The following paragraph sums up the morphology of the Erythrean organism:—'The trypanosome 'is morphologically like *T. brucei* or *T. evansi*, but is not more than '24μ long; free flagellum, which is fairly long, included. It is 'extremely motile, like *T. vivax* of Ziemann. This trypanosomiasis 'appears to be very virulent for ruminants, and thus differs from 'typical surra. In many respects it resembles the disease described 'by Cazalbou in French Sudan under the name Souma. There is no 'tsetse in the infected area, and the suspected fly is a *Tabanus* or a '*Hippobosca*.'

It appears to us that these two species are incapable of absolute diagnosis, save, perhaps, after a rigorous critical comparison. This to us in Africa is impossible, and we have consequently associated our second cattle trypanosome with the senior member. We would add that Broden[4] has also been struck by the unusual similarity between *T. cazalboui* and *T. vivax*, which he considers he has obtained in the Congo.

KAMBOLE, NORTH EASTERN RHODESIA,
October 5th, 1908.

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EXPERIMENTS ON THE COMBINED ATOXYL - MERCURY TREATMENT IN MONKEYS INFECTED WITH *TRYPANOSOMA GAMBIENSE*

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Prolonged experience of Sleeping Sickness treatment in man, and to a certain extent in experimental animals, has proved beyond doubt that Atoxyl by itself effects a really permanent cure only in comparatively few and exceptionally favourable cases of Sleeping Sickness in man, and if administered over a very prolonged period. Nearly all experiences with horses and cattle infected with the different strains of pathogenic trypanosomes lead necessarily to the conclusion that Atoxyl alone is insufficient for a successful issue of the treatment, but only prolongs life to a certain extent, and that finally the animal nearly always succumbs to the disease.

Laveran's[1] experiments showing that a combination of Arsenious Acid and Trypanred was superior on small animals to the use of either drug by itself, led the way for further advance in the treatment of Trypanosomiasis. Thomas and Breinl[2] after the introduction of Atoxyl recommended the combination of Atoxyl and Trypanred, the latter being then, after Arsenic, the best trypanocide known.

The idea of a combined treatment in Trypanosomiasis was taken up later by Moore, Nierenstein and Todd,[3] and their extensive study of the experimental treatment of Trypanosomiasis was directed by the assumption that 'perhaps the recurrence in cases of trypanosome infection after treatment with Atoxyl might be due to some resistant stage of the parasite which survived the first treatment of Atoxyl,

and later gave rise to recurrences of trypanosomes more or less refractory to Atoxyl treatment.' The Atoxyl was, therefore, followed by a second drug; the best results, experimentally, were obtained by using mercury salts, after the disappearance of the parasites from the peripheral circulation had been brought about by Atoxyl. In small animals, such as rats and rabbits, infected with *T. brucei*, the results were far superior to treatment by Atoxyl alone; in large animals, as donkeys, on the other hand, the combined treatment with Atoxyl and Mercury was not found to be efficacious enough to be of practical value.

Plimmer and Thomson[4] repeated the experiments on rats infected with Surra and Ngana, obtaining similar results to those of Moore Nierenstein and Todd, but using different mercury salts. In their hands the combination of Atoxyl and Succinamide of Mercury gave the best results. In a further communication,[5] however, they state that, in small animals at any rate, Mercury has not given altogether satisfactory results, which fact they attribute to the small doses of Mercury. On enlarging the doses of Mercury, chronic kidney, and, to a lesser degree, liver lesions, were observed. They conclude, therefore, that more favourable results might be obtained in dealing with a more chronic trypanosome disease, such as Sleeping Sickness in man.

Laveran's and Thiroux's[6] observations on the combined treatment of Atoxyl and Bichloride of Mercury on Surra in guinea-pigs are, although not very good, superior to the treatment by Atoxyl alone.

According to Uhlenhuth, Hübner and Woithe,[7, 8] the combined treatment on rats infected with Dourine was of considerable value. Criticising Laveran's and Thiroux's experiments, they remark that in guinea-pigs, Atoxyl treatment in general does not give good results, this being due to the fact that these animals very often die after one injection of Atoxyl without any apparent reason.

The good results obtained by the combined treatment of Atoxyl and Mercury in experimental animals, justified its use in human Trypanosomiasis. The Segregation Camps for the medical treatment of Sleeping Sickness in Uganda, gave this method a thorough trial.[9]

Mercury was administered in different forms and at different

intervals; in one series of cases concurrently with the Atoxyl, in another series some time after the last injection of Atoxyl. It is of interest to compare the different modes of administration and the results obtained either with Atoxyl alone or with Atoxyl followed by Mercury. The following methods have been used since the camps were started:—

I. ATOXYL ONLY

(*a*) 0·4 gm. every twentieth and twenty-first day.
(*b*) 0·4 gm. every tenth and eleventh day.
(*c*) 0·4 gm. increasing gradually every tenth and eleventh day up to 0·7 gm.
(*d*) Van Campenhout's method (very similar to method (*c*)).
(*e*) 1 gm. every fifteenth and sixteenth day.
(*f*) 0·6 gm. every fifteenth and sixteenth day.

II. ATOXYL AND MERCURY

Course of Atoxyl treatment lasting a month or six weeks, during which time at least 4 gm. of the drug were given, followed by—

(*g*) Mercury perchloride, $\frac{1}{16}$ grain, twice daily (Dr. J. Collyns).
(*h*) Mercury perchloride, $\frac{1}{6}$ grain, hypodermically, for six doses spread over fourteen days (Dr. Collyns)
(*i*) Metallic Mercury (Lambkin's cream), 5 minims once a week (Dr. C. J. Baker).
(*j*) Intramuscular injections of 1 c.c. of a 1 per cent. solution of soluble mercury salts, repeated every five days (Drs. Goodliffe and Bayon).

Combined simultaneous Atoxyl and Mercury treatment (Dr. van Someren)—

(*k*) First day, Atoxyl, 1 gm.; Mercury perchloride, 0·01 gm. Second day, Atoxyl, 1 gm. On fourteenth day, Atoxyl, 0·5 gm.; Mercury perchloride, 0·01 gm. On fifteenth day, Atoxyl, 0·5 gm., repeating every fourteenth and fifteenth day.
(*l*) Same as above, except that one initial dose of 1 gm. Atoxyl is given, the remaining doses being 0·5 gm.

A comparison of the results obtained at the various camps with Atoxyl, and Atoxyl and Mercury treatment, during the period December, 1906, to November, 1907, is seen in the following table (Table VII, p. 8, of the report):—

Present State on February 29, 1908.	Busiro. (Dr. Collyns.)		Kyagwe. (Dr. van Someren.)	Usoga. (Dr. C. J. Baker.)	
	Atoxyl only. Method (*b*) (*c*) (*d*)	Atoxyl and Mercury. Method (*g*) or (*h*)	Atoxyl and Mercury. Method (*k*) or (*l*).	Atoxyl only. Method (*b*), few (*e*)	Atoxyl and Mercury. Method (*i*)
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Improved	8	19	40·5	35	30
Relapsed	6	15	11	7	5·5
Continue in same state ...	38	62	12·5	3	40
Absent at time of examination	3	—	21	20	—
Died	45	4	15	35	25·5
Number of cases ...	382	100	328	252	73

The following table shows the comparative results from November-February, 1908, wherein the state of the disease is classified. A, meaning very early cases without symptoms, except gland enlargement; B, early cases with symptoms, itchy skin, &c.; C, advanced cases; D, very advanced cases (Table XVIII, p. 19, of the report).:—

Present State on February 29, 1908.	Class of Case on Admission.							
	A		B		C		D	
	Atoxyl only.	Atoxyl and Mercury.	Atoxyl only.	Atoxyl and Mercury.	Atoxyl only.	Atoxyl and Mercury.	Atoxyl only.	Atoxyl and Mercury.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Improved ...	42	60·5	35	63	24	41	7	12
Relapsed ...	1	3	4	4	3	7	—	—
Continue in same state	50	23	46	23	56	36	53	44
Absent at time of examination	3	10	5	8	5	6	8	—
Died	4	3·5	10	2	12	10	32	44

The method of treatment according to 'improved' cases is, therefore:—

I.	Method (*k*), 64 per cent.	Atoxyl and Mercury.
II.	Method (*j*), 58 per cent.	
III.	Method (*g*), 39 per cent.	
IV.	Method (*i*), 34·5 per cent.	
V.	Method (*b*), 34 per cent.	Atoxyl alone.
VI.	Method (*b*), 32 per cent.	
VII.	Method (*f*), 10 per cent.	

Bohne[10] describes one case of trypanosome fever which improved markedly under the combined Atoxyl-Mercury treatment, but the time of observation is too short to conclude that this patient has been definitely cured.

Broden and Rodhain,[11] on the other hand, come to the conclusion that the combined treatment did not prove superior at all to a treatment with Atoxyl alone.

As Moore's, Nierenstein's and Todd's work was mostly carried out on animals infected with *T. brucei*, it seemed advisable to repeat the work on monkeys infected with *T. gambiense*. Six animals (*Cercopithecus callithricus*) were inoculated with our laboratory strain of *T. gambiense*, and after varying time subjected to treatment. Acetylated Atoxyl, which, in former experiments, had proved itself less toxic than Atoxyl, was used.

EXPERIMENT I.—*Cercopithecus callithricus*, ♀, weight 2 k. 700 gm., was inoculated on April 28th with *T. gambiense*. At the time of the third relapse, on June 1st, treatment was begun after the animal had shown typical signs of the infection; the animal was markedly anaemic, and had lost over 300 gm. in weight. On June 1st and 2nd, an injection of 0·1 gm. of acetylated Atoxyl was given. The parasites disappeared after the first injection. On June 10th and 18th, the Atoxyl was followed by an intra-muscular injection of 0·05 gm. of Sublimate. On July 2nd and 3rd, the Atoxyl injection, and on July 15th and 16th the injections of Sublimate were repeated. Up to November, daily examinations of the blood were made, and since then the peripheral blood has been examined twice weekly. No trypanosomes have been seen since June 1st, the day of the injection. The blood count is normal (March 7th). The animal has increased in weight to 2 k. 845 gm., which has been maintained with slight variations since the middle of November.

EXPERIMENT II.—*Cercopithecus callithricus*, ♀, weight 2 k. 700 gm., was inoculated on April 28th with *T. gambiense*. The animal never showed parasites in great number. On June 16th, treatment was begun, as the symptoms of the disease became more and more marked. On June 6th and 10th, 0·1 gm. of acetylated Atoxyl was administered; these injections were followed on June 18th and 19th by 0·05 gm. of Sublimate, intra-muscularly, in the gluteal region. The second injection of Sublimate was followed by complete paralysis of the hind leg,

which was most probably due to an injury of the sciatic nerve through the injection. This paralysis, however, passed off very gradually, and after two and a half months the mobility of the leg was normal. On July 2nd and 3rd, the acetylated Atoxyl injections were repeated, and on July 15th and 16th the intra-muscular injections of Sublimate. The animal is still alive, and parasites have never been seen since the first inoculation. The blood is normal, and the weight is at present 2 k. 895 gm.

EXPERIMENT III.—*Cercopithecus callithricus*, ♀, weight 3 k. 600 gm., was inoculated on April 28th, 1908, with *T. gambiense*. Treatment was started on June 6th, the time of the third relapse. 0·15 gm. of acetylated Atoxyl was administered. This injection was repeated on June 10th, and followed on June 18th and 19th by intra-muscular injections of 0·075 gm. of Sublimate. The Atoxyl injections were repeated on July 2nd and 3rd, and the Sublimate injections on July 15th and 16th. This animal is still alive; parasites have never been seen since the treatment was started. The blood count is at present normal, and the weight, which had increased by July 21st to 3 k. 850 gm., has remained constant since November at 3 k. 600 gm.

EXPERIMENT IV.—*Cercopithecus callithricus*, ♂, weight 1 k. 800 gm., was inoculated on April 28th. Treatment was started on June 3rd with injections of 0·1 gm. of acetylated Atoxyl, followed by an administration on June 8th of 0·01 gm. of Sublimate in pill form, by the mouth. A very severe attack of diarrhoea, which lasted from June 9th—12th, followed this medication; the stools were slimy and haemorrhagic. On June 18th and 19th, 0·1 gm. of acetylated Atoxyl was given, followed on June 23rd by one injection of 0·05 gm. of Sublimate. On July 2nd and 3rd, 0·1 gm. of acetylated Atoxyl was given, followed on July 15th and 16th by 0·01 of Sublimate, intra-muscularly. The Sublimate injections were not followed by any untoward effects. The animal is still alive; parasites disappeared after the first injection of Atoxyl, and have never been seen since. The blood is normal, and the weight 2 k. 100 gm.

EXPERIMENT V.—*Cercopithecus callithricus*, ♀, weight 1 k. 900 gm., was inoculated on April 28th. At the time of the second relapse on June 3rd, treatment with acetylated Atoxyl was begun. Two injections of 0·1 gm. on June 3rd and 4th were followed by an administration of 0·1 gm. of Sublimate in pill form by the mouth. In this case, as in Experiment IV, a very severe diarrhoea with slimy and haemorrhagic stools occurred, which, however, passed off in a few days. The Atoxyl injections were repeated on June 18th and 19th, followed on June 26th and 27th by an intra-muscular injection of 1 c.c. of Donovan's solution. On July 2nd and 3rd the Atoxyl injections were repeated, followed on July 15th and 16th by an injection of 1 c.c. of Donovan's solution. The parasites disappeared from the peripheral circulation of the animal after the first injection, and were never seen again. The animal was found dead on July 17th. The post-mortem showed fatty degeneration of the heart; the spleen was slightly enlarged, of firm consistence, with well-marked hypertrophic malpighian follicles. The kidneys were enlarged and markedly congested, the cortical substance was not defined from the medullary substance, and haemorrhagic stripes intersected the yellow cortical substance. Microscopically, the liver showed typical fatty degeneration of the liver cells; the kidneys showed on section a well-marked parenchymatous nephritis. The death of the animal was due in all probability to Mercury poisoning.

EXPERIMENT VI.—*Cercopithecus callithricus*, ♀, weight 2 k. 750 gm., was inoculated on June 6th with *T. gambiense*. Treatment was started on June 18th with a view to ascertaining whether a short treatment at an early stage can effect a permanent cure. 0·1 gm. of acetylated Atoxyl was given, and the same dose repeated on June 18th. The parasites, which were fairly numerous before treatment, disappeared promptly. In this case, however, the acetylated Atoxyl

produced a harmful effect. The doses which proved perfectly harmless in the foregoing experiments caused the whole complex of symptoms of a typical Atoxyl poisoning. Tremors occurred all over the body, and, at the same time, a severe slimy diarrhoea started. These symptoms, however, passed off in the course of a week. On June 26th and 27th, injections of 1 c.c. of Donovan's solution was given. This animal is still alive, weighing at present 3 k. 100 gm. The blood is normal; parasites have never been seen since treatment was begun.

The foregoing experiments show that in five cases out of six, the administration of Acetylated Atoxyl and Sublimate, and Donovan's solution, in monkeys (*Cercopithecus callithricus*), has effected a complete cure.

If we consider that in our experiments *Cercopithecus callithricus* usually succumb to an infection of *T. gambiense* in the course of 40-60 days, the results of Atoxyl-Mercury treatment in monkeys infected with *T. gambiense* must be looked upon as very encouraging indeed.

The length of time which has elapsed since treatment was discontinued is probably sufficient to permit of the animals being considered as definitely cured of the disease.

Concerning the *action* of the combined treatment by Atoxyl and Mercury, a conclusive opinion can hardly yet be expressed. Mercury has proved beyond doubt to have not the least effect on trypanosomes in the peripheral circulation. It is, therefore, very tempting to accept Moore's, Nierenstein's and Todd's suggestion of its action on a secondary resistant form of the parasite which it destroys; but whether we accept this hypothesis or suppose that the Mercury acts merely as an internal disinfectant after the destruction of the parasites by Atoxyl, the success of the combined treatment seems to depend upon the administration of the two drugs either concurrently or in rapid succession.

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DRUGS FROM THE CONGO

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In a former article* I have described some of the native African Drugs in the Museum of the University of Liverpool and spoken of the importance of a closer study of the remedies which have been in use among the natives, perhaps for centuries, for the treatment of disease.

MOUÏNDU OR MUINDU

On July 19th, 1906, I received from Mr. Robert Newstead a packet of drugs which were brought to this country by Prof. J. L. Todd, and had been sent, together with Palabanda and other drugs from the district of Banana, to the late Dr. J. Everett Dutton, on the 17th February, 1904, by the Commandant Du Camp of the lower Congo, in response to enquiries by Dr. Dutton concerning native remedies against Sleeping Sickness. A further sample of the same drug was given to me in March, 1909, by Dr. J. W. W. Stephens, which had been sent by Dr. E. Etienne from the same district.

Macroscopic Characters of the Drug.

The material consisted of a piece of stem 22 cm. long by 3 cm. in diameter, together with leaf stalks and detached leaves, of the appearance of which fig. 1 gives a good idea.

The cortical portion which was readily removed from the hard woody portion had an astringent taste, followed by a persistent bitterness. No part of the drug had any characteristic odour. The outer layer of the cortical portion was velvety to the touch, longitudinally furrowed, with occasional transverse cracks, and

* Prosper H. Marsden: Notes on the Drugs of West Africa. Quarterly Journal of the Liverpool University Institute of Commercial Research in the Tropics, Vol. III, No. 6.

numerous transverse lenticels. This velvety layer could be easily removed by the finger nail, showing a dark red-brown hard bark beneath.

A transverse section of the stem showed the outer bark to be 1·5 mm. thick and the inner red-brown layer 3 to 4 mm. thick; in this could be seen many white dots, more numerous towards the inner side. The bark had generally a short fracture, with fine fibres showing at the site of fracture.

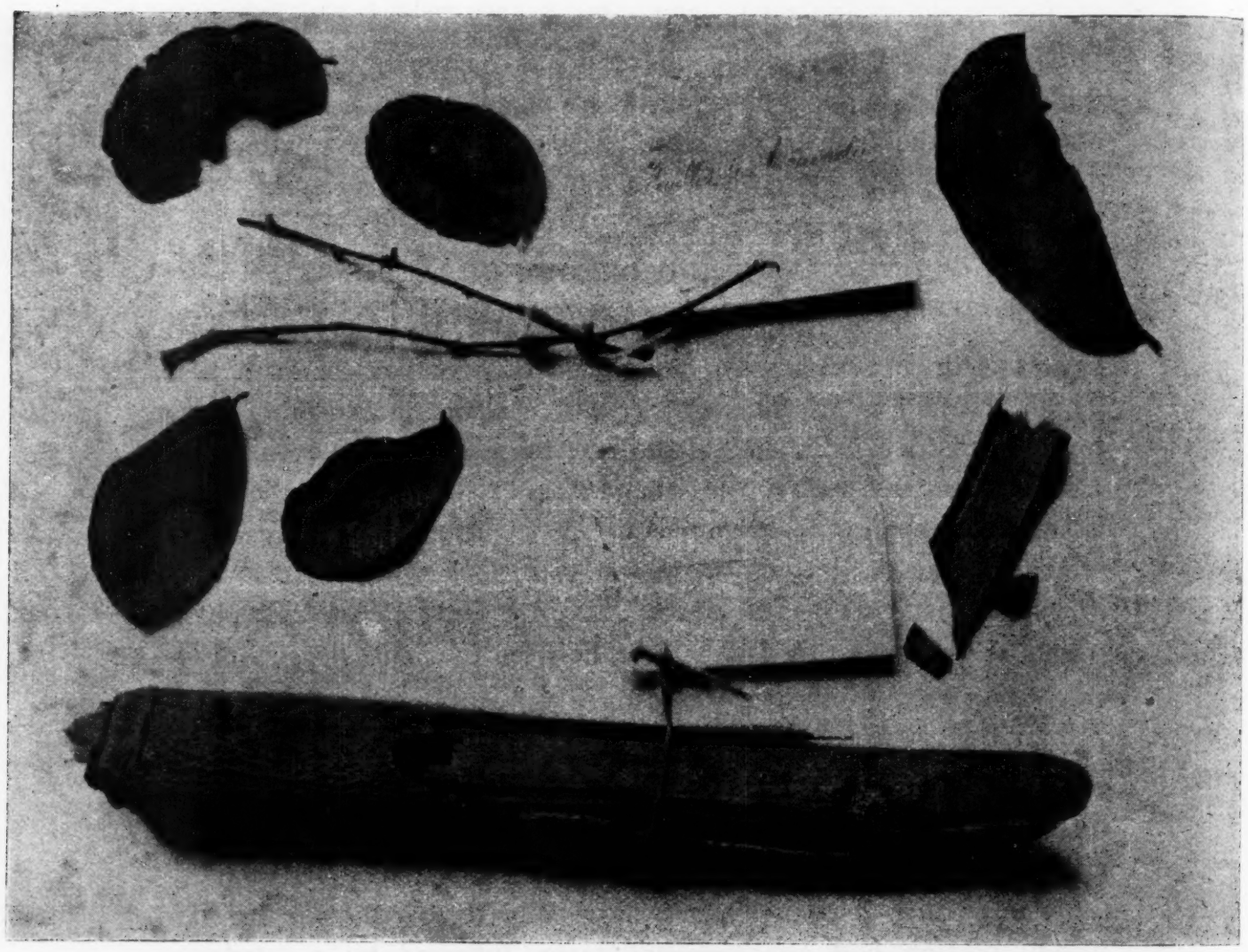

FIG. 1.—Leaves and stem of Muindu.

The wood, 2·5 cm. at the thickest part, was hard, yellowish-white with five rings and minute pith, and with a powerful lens the lacunae could be seen which are characteristic of the genus *Strychnos*.

As the bark of Muindu has some resemblance to that of the false Angostura Bark (*Strychnos Nux Vomica*, Lin.) described about half a century ago,† and both Muindu and Palabandâ have something in common with Sassy Bark (*Erythrophloeum guineense*, Don.), it was thought that in view of the medicinal use of these an examination of Muindu might be of value.

The sample from Dr. Etienne consisted of three pieces of branches of the plant, which he described as a shrub growing in the plains. The largest of the branches was some 50 cm. in length by 2·5 cm. in diameter at the lower end, which had the velvety appearance of the older stem above mentioned. The younger branches were smoother, and had sharp thorns 5 mm. to 2 cm. long irregularly distributed upon them. There were no leaves with this specimen, but the thinnest branch had evidence of leaf scars, and was of the same colour and appearance as the leaf stalk of the Muindu received from Dr. Todd. A note by Dr. Etienne states that the natives scrape off the liber and macerate in treaty rum. Of this preparation a few drops are instilled into the eye for Sleeping Sickness.

A tincture was prepared from the bark by maceration in 60% alcohol of a strength of one drug in ten of menstruum. This was tested on frogs by Dr. C. O. Jones in the Department of Bio-Chemistry of the University with negative results.

This tincture was shaken out with ammoniated chloroform three times, and the chloroformic layer being allowed to evaporate to dryness gave a residue which upon treatment with sulphuric acid and potassium bichromate failed to give the usual reaction for strychnine.

Microscopic characters of the Bark of Muindu.

A transverse section of the bark showed that the velvety layer had an indefinite appearance.

The suber is formed of flattened thin-walled cells, the cortical parenchyme consists of polygonal cells having thin walls and intercellular spaces, and containing a brown material which gave the reaction for tannin with ferric chloride.

Although, as above mentioned, I was unable to obtain the reaction for strychnine from the tincture of Muindu, upon flooding a transverse

† Planchon et Collin. Les drogues simples d'origine végétale, Vol. I, p. 662.

section of the bark with sulpho-vanadic acid the customary violet coloration due to strychnine was afforded. This was not given by a similar section which had been well washed with an alcoholic solution of tartaric acid previous to irrigation with sulpho-vanadic acid.

In the parenchyme are found a number of groups of sclerenchyme with small lumen. These groups are more numerous towards the inner side, where they assume a quadrangular shape and are arranged very closely together (fig. 2). In longitudinal section these schlerenchymatous fibres are seen to be elongated longitudinally (fig. 3).

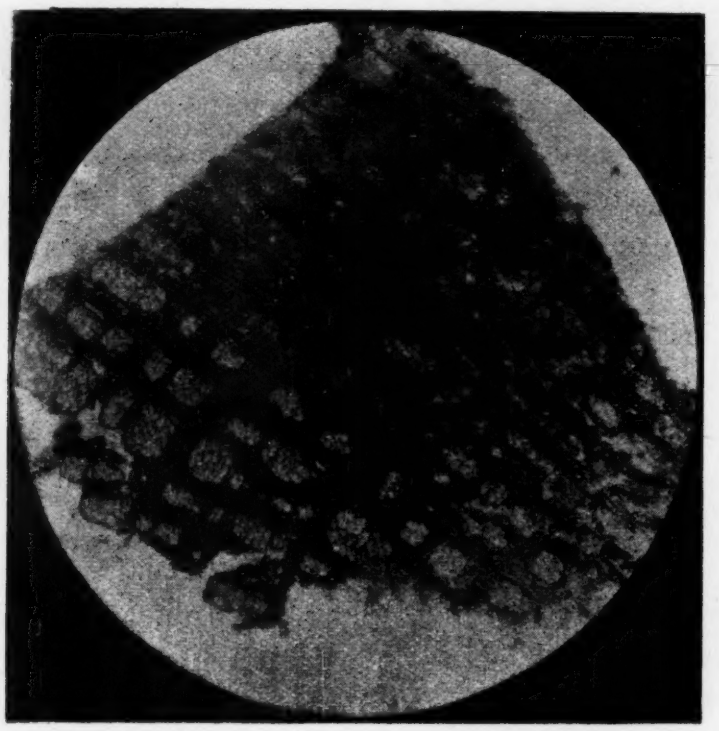

FIG. 2.—Transverse section of bark of Muindu.

Characters of the leaf of Muindu.

The leaves have not the character of those of the genus *Strychnos*, but as they were detached when received, it is possible that they are not from the same plant as the stem and branches. A photomicrograph of the mid-rib of one of the leaves is seen in fig. 4, but in view of the statement of Dr. Etienne that the bark of the plant was the part used in medicine, the leaves were not further investigated.

FIG. 3.—Longitudinal section of bark of Muindu.

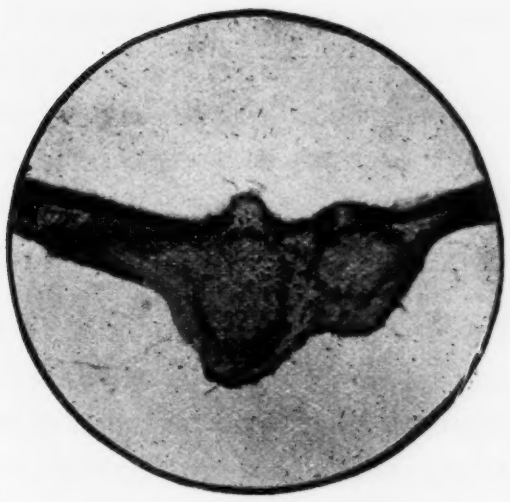

Fig. 4.—Transverse section of mid-rib of leaf of Muindu.

A GREGARINE PARASITIC IN THE DOG-FLEA, *CTENOCEPHALUS SERRATICEPS*

BY

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The excuse for describing this parasite which inhabits the alimentary tract of the dog-flea must be in the fact that much work is now being done with fleas generally, and some confusion may be saved to others, working at these insects, if some details of its various phases are related.

This parasite was found, in varying numbers, in the fleas of two fox-terrier dogs, mother and son, which had lived in Port Said nearly all their lives. Its stages follow the well-known gregarine type frequently found in Nematodes and Culicids. It is a Cephalin showing a well-marked cycle of sporogony, all the stages of which are completed within the body of the flea, its host; while, like many other gregarines, the cycle of schizogony, so far as can be found out, is wanting.

It is convenient, for the sake of description, to begin with that phase of the cycle which is first seen in the flea.

I. THE EARLY TROPHOZOITE

This stage, as in similar Gregarinidae found in the Worms, Echinoderms, and Ascidians, is the first resting phase of the parasite in the flea. It is probably caused by the direct infection of a stomach cell by a sporozoite derived from the rupture of the sporocyst, the sporozoite having been eaten by the flea larva when crawling on the dog's back. But it is possible that it may be derived from the merozoite of a cycle of schizogony which has taken place either outside the host or within the stomach of the flea-larva; up to the present time, however, no traces of such cycle have been noted.

The early trophozoite, then, is a small circular cell embedded between the pyriform epithelial cells lining the stomach of the flea. As many as twenty-five may be found in one flea situated near the proventriculus, but occasionally near the pylorus. They are frequently found in pairs. These cells contain large refractile granules, and a readily staining nucleus. The whole cell stains before the stomach cells when the organ containing it is placed upon agar having an aniline dye in suspension. In clear specimens a slender process can be detected, by which the parasite is attached to the remains, probably, of its trophic cell that has been destroyed by it. The relation of this early form to the rostrated trophozoite next to be described is not merely conjectural, because it is only found in young infected fleas and in those containing older trophozoites which cannot be mistaken. Besides, the granules in all the early stages are very characteristic. The fact that these early forms are only found in young flea imagines makes it highly probable that this parasite is ingested by the larva, for in older fleas the more highly developed phases of sporogony only are to be found, and it is unusual to see parasites in other than contiguous stages in the same flea. The age of a flea may be roughly estimated by the degree of growth of the ova in the ovisacs in the females, and by the degree of development of the spermatozoa in the vesiculae seminales in the males. In the dog-flea the absence of spermatozoa in the spermatheca of the female is almost certain evidence of the extreme youth of the imago, because fecundation by the male takes place very soon after the metamorphosis of the female is completed; and in the male the spermatozoa within the vesiculae seminales are tied together by their heads into compact bundles very soon after the imago has hatched from the nymph.

II. THE TROPHOZOITE AND ITS DEVELOPMENT

During this phase the parasite develops a well-marked epimerite. In its growth it may reach a size equal to ten times that of a stomach epithelial cell. It is pear-shaped, and is fixed by its epimerite to the lining membrane of the stomach. The body of the cell is divided, in the early stages, by a horizontal septum into two nearly equal halves, the protomerite and the deutomerite. As the cell grows it becomes

more highly granular and consequently darker to transmitted light, and the septum less distinct. It also begins to lose its pyriform shape, and slowly reverts to its circular form. When full grown it is circular and the horizontal septum has vanished, while it is full of large refractile granules and the epimerite has disappeared. These trophozoites are usually found in pairs within the stomach and adhering to its wall.

III. THE FORMATION OF THE SPORONT

The epimerite or rostrum has completely disappeared, and the parasite is circular. The next phase seen of this particular parasite is that of the association and encystment of two sporonts. The cyst is embedded in the stomach wall, and consists of a thick fibrous capsule. The gametes appear as small granules. The analogy of similar sporonts parasitic in other members of the Arthropoda shows that they are formed in the following manner:—A male and female sporont conjugate and become encysted in their mother tissue A nuclear spindle is formed from a small portion of the nucleus of the sporont and divides, the remainder of the nucleus degenerating. The spindle then produces daughter nuclei by mitosis and they again divide, until a number of nuclei are formed which bud off the surface of the sporont. Each budded nucleus is then the primary sporoblast, and ultimately becomes the male or female gamete according to the sporont from which it was originally derived.* Whether in this instance, the male gamete becomes flagellated or not, it is impossible to say, but in the specimens examined in this stage no flagella were ever seen; however, the cephalonts are very difficult to stain, as they are surrounded with such a thick fibrous wall. The males are said to burst their way into the female half of the cephalont, and there fertilise the female gametes.

The cephalont is a very remarkable looking cell embedded in the stomach wall. It is large, frequently thirty times the size of a stomach epithelial cell, and its two granular, male and female, halves make it very characteristic and distinct. It can be rolled when the stomach is pressed under the cover-slip, but I have not succeeded in staining the gametes, as sufficient force to rupture the cephalont causes their destruction.

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* I am indebted to Professor Minchin's article on the Sporozoa, in Lankester's Zoology for the description of this process.

IV. THE FORMATION OF THE OOCYST

After the fertilisation of the female gametes the male half of the cephalont degenerates, and the female portion grows until it fills the whole cell, which then separates from its attachment to the stomach wall and becomes free in the cavity. Each female gamete grows in size after fertilisation, while the fibrous cell-wall thins with the distension. The gametes, or zygotes as they must now be called, are highly refractile bodies, each about the size of a red-blood corpuscle. They are packed tightly in the fibrous capsule or sporocyst, which resembles a pomegranate that has been cut through the middle with a sharp knife. It is about fifty times the size of a stomach cell, and if the stomach wall be ruptured it may be extruded, transferred to a clean slide, burst, stained and examined. But under natural conditions it passes down to the pylorus, through which it is too large to move, and it ruptures, and the contained zygotes escape into the intestines (Malpighian tubes) under the influence of the peristaltic action.

V. THE DEVELOPMENT OF THE SPOROBLASTS

If a cyst be expressed from the stomach, crushed on a slide, and the sporoblasts stained by any method giving the Romanowski result, they will be found to be lanceolate in shape but of somewhat irregular contour. Each one has some chromatin, but this varies in amount from a minute dot to an extensive streak. The cell-wall is glistening, and appears almost chitinous, while there is some very feebly staining cytoplasm. But they have a high osmotic index, for if the intestine containing them be placed unruptured upon agar spread on a glass slide and containing polychrome methylene blue, according to the method described in the Journal of Physiology, for September 16th, 1908, by H. C. Ross, it will be noticed that the living sporoblasts accept the stain before the cells forming the lining to the tube. But even these cells accept the stain more readily than the hepatic cells, or the epithelial cells of the stomach or of the salivary glands. They are, therefore, very susceptible to external influences.

After the cyst has ruptured the sporoblasts pass into the intestine, where they undergo a still further change. They become barrel-shaped sporocysts; the contour is regular, and in the fresh

state they are yellow and roll up and down the Malpighian tube with the flow of its contents. Sometimes they are very numerous, and can be seen at once on examining the intestines.

VI. THE FORMATION OF THE SPOROZOITES

While in the intestine the barrel-shaped sporocyst gives rise, in its interior, to eight rod-shaped sporozoites. These are at first found tied together by their ends like a bundle of cigars or bananas, so that when viewed from above the sporozoite gives the appearance of eight small separate circles within a circle. Up to the present it has been impossible to separate these sporozoites or to stain them, but when the sporocyst passes into the rectum of the flea it ruptures, and the sporozoites are set free with the faeces. The ultimate destination of the sporozoites has not been traced, but they are passed with the faeces. Whether, as stated before, there is a further cycle of schizogony, or whether the sporozoites are eaten directly by the flea larva and a new cycle of sporogony started, it is not possible to say. But the flea larva is difficult to obtain, and still more difficult to dissect. During two years' work with dog fleas the number found infected with this parasite was 38 per cent.

I suggest as a name for this Gregarine, *Gregarina ctenocephali canis.*

THE ACTION OF ARYL-STIBINIC ACIDS IN EXPERIMENTAL TRYPANOSOMIASIS

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Plimmer's and Thomson's important discovery of the trypanocidal action of Antimony, an element chemically closely allied to Arsenic, was the starting point for extensive investigations on the value of different Antimony compounds in the treatment of Trypanosomiasis. In their first experiments[1] they used Potassium antimonyl tartrate, and observed that this drug destroyed the trypanosomes in the peripheral circulation more rapidly than Atoxyl. The injections caused neither pain nor inflammatory changes of the tissue. In their experiments, out of twenty-five rats only four showed recurrences; nine lived for over two hundred days, and nine considerably over one hundred days; the remaining three died without any symptoms of Trypanosomiasis, and in none were trypanosomes found after death.[2]

Mesnil and Brimont[3] were able to confirm Plimmer's and Thomson's observations concerning the powerful trypanocidal action of Potassium antimonyl tartrate. Their experiments on several strains of pathogenic trypanosomes, however, proved that after one injection the parasites usually reappeared, sometimes after a very short time. In Ngana infected rats, as many as nine relapses were observed, and the drug had always the same transitory effect; most of the animals died finally either with or without parasites after discontinuation of the treatment. Their results in animals infected with *T. evansi*, were more satisfactory; and it is specially noteworthy

that an Atoxyl-resistant Surra strain reacted to Antimony in the same way as a normal strain. A preventative action was only noticed if the drug and the parasites were injected simultaneously in two different distant places.

In our hands,[4] Sodium antimonyl tartrate did not give such promising results in the treatment of rats, a fact which might be due to the use of an especially virulent strain of *T. equiperdum.* One horse injected with a strain of cattle trypanosomes brought back from the Congo, and one donkey infected with an Atoxyl-resistant strain of Ngana, were treated with Sodium antimonyl tartrate in fairly large doses. In both cases the drug caused the prompt disappearance of the parasites; the interval between the relapses, however, became shorter and shorter after each injection, and finally both animals succumbed to the disease.

Manson[5] was the first to administer Sodium antimonyl tartrate to a case of Sleeping Sickness. As Atoxyl given in large doses did not seem to have any effect on the trypanosomes, a treatment of Antimony in small doses was begun. It caused the disappearance of the parasites, but eighteen days afterwards parasites were again seen in the peripheral circulation. As the injection had caused intense irritation and pain, two grains of Antimony were given by the mouth; this was followed by nausea, and seemed to increase the mental depression of the patient. Antimony treatment was discontinued, and Atoxyl again given.

For further experiments Mesnil and Brimont[6] used mice infected with different laboratory strains of pathogenic trypanosomes. According to their results they were able to separate the strains into two groups. The parasites of the first group disappeared after a single injection: Surra and Dourine belonging to this group. In the second group, to which all other strains belong, the parasites disappeared after the injection of Tartar emetic, but only to reappear within a few days. The negative phases after each injection became shorter and shorter, and the animals finally succumbed to the disease.

In the discussion, Laveran[7 a, b] states that in his hands, Tartar emetic did not prove very satisfactory in guinea-pigs, as after the rapid disappearance of the parasites following the first injection, relapses occurred very frequently. Sulphide of Antimony, in his experience, is much less active than Sulphide of Arsenic (orpiment).

Uhlenhuth and Woithe[8] repeated the experiments with Sodium antimonyl tartrate on twenty-seven rats infected with *T. equiperdum*, but their results were as discouraging as those with Arsenious acid. Even repeated injections of 0·003—0·005 gramme of the Sodium salt and 0·002—0·003 gramme of the Potassium salt were unable to effect even temporary disappearance of the parasites from the blood.

Broden and Rodhain[9] used soluble as well as insoluble compounds of Antimony in Sleeping Sickness treatment. The hypodermic injections caused great irritation and pain, and were followed, even when given intramuscularly, by large swellings; this reaction persisting for some days, and only disappearing after one week. Therefore, the drug was administered intravenously. A dose of 0·07 gramme was sufficient in some cases to cause the parasites to disappear, but they very soon reappeared in the peripheral circulation. These observers recommend a dose of 0·1 gramme of Tartar emetic. This dose given intravenously did not usually cause any severe symptoms; it was followed sometimes by profuse perspiration and vomiting. After repeated injections the patients usually lost their appetite and complained of general malaise. On interruption of the treatment these symptoms passed off. They were able to confirm the rapidity of the destructive action of Antimony on the parasites, but remark that, 'Ces constatations doivent nous imposer une extrème reserve dans l'appréciation de la valeur de l'antimonie dans le traitement de la trypanosomiasis humaine et éxigeront une experimentation longue et patiente.'

They, however, place, for the present at least, the soluble Antimony compounds on the same level as Atoxyl.

Broden and Rodhain, as well as Martin and Darré,[10] combined the Atoxyl treatment of Sleeping Sickness patients with injections of soluble Antimony compounds with very encouraging results.

Good results of Antimony treatment in experimental Syphilis are recorded by P. Salmon,[11] and Broden and Rodhain[9] were able to confirm the beneficial effect of Antimony in human Syphilis.

Plimmer's and Thomson's observations on the rapid action of Potassium antimonyl tartrate on trypanosomes in the blood of infected animals seemed to justify an attempt to prepare an organic Antimony compound analagous to Atoxyl. Moreover, a comparison of the effects of injections of Sodium arsenate with those of Atoxyl made it

probable that injections of the Sodium salt of Amino-phenyl-stibinic acid would be far less irritating and permit the introduction of a larger amount of Antimony into the organism without toxic symptoms.

After many unsuccessful attempts, we succeeded in preparing the *p.m.* and *o.* amino-phenyl-stibinic acids. The action of the *p.* and *m.* compounds has been studied on experimental animals infected with various laboratory strains of pathogenic trypanosomes; the *o.* compound was, after a few tentative experiments, given up as impracticable.

I. EXPERIMENTS WITH *m.* AMINO-PHENYL-STIBINIC ACID

m. amino-phenyl-stibinic acid was used in the form of its acetylated derivative. This latter proved itself in the first experiments the less toxic, and produced no appreciable irritation at the site of inoculation, whereas the *m.* compound caused marked swellings and abscesses.

A. Rats.

Medium-sized rats of the weight of 180-220 grammes were used for the following experiments. Untreated animals succumbed to an infection of *T. brucei* in 4-6 days on the average. Treatment was usually started when numerous parasites were present in the peripheral blood. In a few experiments the compound was injected only at a late stage, some hours before death; but then the animal always died from the infection. The toxic dose was found to be 0·75 c.c. of a 5% solution; an injection of 0·5 c.c. of the same solution corresponding to 0·025 gramme of the drug was usually well borne. No abscesses were noticed at the site of injection. After the administration of this dose the parasites disappeared from the blood in 12-16 hours. Smaller doses were only able to effect a disappearance of the parasites from the blood, when repeated. The parasites, however, often reappeared after a comparatively short time; a further injection of the drug again resulting in the disappearance of the parasites.

Table I shows the details of the result of treatment on thirty-three rats: only one rat is still living after 136 days.

TABLE I.

Exp. No.	Disease	Date of inoculation	Treatment at the end of—	c.c. of 5 % solution	Relapse after	Remarks
282 A.	*T. brucei*	Oct. 20	2, 3, 8, 23 days	2 c.c.	—	Lived 93 days. Abscesses of lung.
B.	,,	,,	2, 3, 8 days	1·5 c.c.	—	,, 10 ,, Kidney lesions; spleen small.
288 A.	,,	Oct. 26	2 days	0·75 c.c.	—	,, 5 ,, Antimony poisoning.
B.	,,	,,	,,	0·75 c.c.	—	,, 5 ,, ,, ,,
293 A.	,,	Oct. 30	2, 13 days	1 c.c.	—	,, 19 ,, No signs of Trypanosomiasis.
B.	,,	,,	,,	1 c.c.	—	,, 59 ,, ,, ,,
254 A.	,,	Oct. 17	3 days	1·25 c.c.	—	,, 4 ,, Late stage of infection. Tryps. still present at death.
B.	,,	,,	,,	1·25 c.c.	—	,, 4 ,, ,, ,, ,,
C.	,,	,,	,,	1·25 c.c.	—	,, 4 ,, ,, ,, ,,
D.	,,	,,	,,	1·25 c.c.	—	,, 4 ,, ,, ,, ,,
255 A.	,,	Oct. 17	3 days	1 c.c.	—	,, 4 ,, Late stage of infection. Tryps. present at death.
B.	,,	,,	,,	1 c.c.	—	,, 4 ,, ,, ,, ,,
C.	,,	,,	,,	1 c.c.	—	,, 4 ,, ,, ,, ,,
D.	,,	,,	,,	1 c.c.	—	,, 4 ,, ,, ,, ,,
256 A.	,,	Oct. 22	3 days	—	—	Died before injection.
B.	,,	,,	,,	0·6 c.c.	—	Lived 4 days. Late stage of infection. Tryps. present at death.
259 A.	,,	Oct. 28	2, 14, 25 days	1·25 c.c.	25, 30 days	36 days. Tryps. present at death.
B.	,,	,,	2 days	0·5 c.c.	—	3 days.
262 A.	,,	Nov. 11	3 days	0·3 c.c.	—	3 days. Treatment started 2 hrs. before death. Tryps. present at death; slightly decreased in number.
B.	,,	,,	,,	0·3 c.c.	—	,, ,, ,, ,,
C.	,,	,,	,,	0·3 c.c.	—	,, ,, ,, ,,
D.	,,	,,	,,	0·3 c.c.	—	,, ,, ,, ,,
263 A.	,,	Nov. 12	2, 3, 9, 11, 12, 18 days	0·5 c.c.	9, 18	25 days. Blood swarming with Tryps. at death.
B.	,,	,,	,, ,,	0·5 c.c.	—	4 days. Tryps. absent at death.
264 A.	,,	Nov. 12	2, 3, 9, 11, 18 days	1·75 c.c.	18	22 days. Blood swarming with Tryps. at death.
B.	,,	,,	,, ,,	1·75 c.c.	—	Animal living after 136 days.
183	*T. gambiense*	June 29	38, 136 days	1·0 c.c.	85, 135	140 days. Tryps. absent at death.
257 A.	,,	Oct. 28	16, 17, 26, 27 days	1·05 c.c.	47	62 days. Tryps. present at death.
B.	,,	,,	16, 17 days	0·5 c.c.	—	20 days. Tryps. absent at death; spleen enlarged, kidneys normal.
C.	,,	,,	16, 17 days	0·5 c.c.	22	23 days. Tryps. present at death.
D.	,,	,,	16, 17 days	0·5 c.c.	—	18 days. Tryps. absent at death.
E.	,,	,,	16, 17, 26, 27 days	1·05 c.c.	—	32 days. ,, ,,
F.	,,	,,	16, 17, 26, 27 days	1·05 c.c.	—	28 days. ,, ,,

B. DOGS.

Two dogs were used for the experiments (see Table II). In experiment 245 the dog was treated with Sodium-amino-phenyl-stibinic acid; in experiment 244 with the acetylated derivative of the same compound. In the former experiment severe abscesses resulted from the injections, and after a very short time the animal succumbed to the toxic effects of the drug. The post-mortem examination revealed a haemorrhagic nephritis.

The subcutaneous injections of the acetylated compound, on the other hand, did not cause any irritation. Only frequently repeated large doses effected a disappearance of the parasites from the blood. Very soon, however, trypanosomes reappeared again. The animal succumbed to a severe toxic haemorrhagic nephritis due to the Antimony.

C. RABBITS.

Six rabbits were inoculated with *T. brucei*, and after the disease had become well established treatment was begun. But even prolonged administration of fairly large doses—0·1 gramme per injection—was not able to cope with the disease, and all the animals succumbed to the infection. Although parasites were very rarely seen in the peripheral blood, the well-known symptoms of a Ngana infection were more or less pronounced during the whole course of the treatment.

D. GUINEA-PIGS.

Treatment was only attempted in the case of three guinea-pigs, as it was soon apparent that these animals did not bear well, effective doses of Antimony. It was found that if this drug was administered in sufficiently large doses to destroy the parasites, the animal died from severe kidney lesions; small doses, on the other hand, did not, even if administered repeatedly, have a noticeable effect on the parasites in the blood.

It was noticed that on standing the *m.* amino-phenyl-stibinic acid lost its action on trypanosomes, and caused on injection serious toxic symptoms. This fact was due to a decomposition of the compound into Aniline and Antimonic acid.

TABLE II.

Exp. No.	Disease	Weight	Date of inoculation	Treatment at the end of—	Amount injected	Relapse after	Remarks
244	*T. brucei*	9 k. 600 grs.	Aug. 7	4, 5, 6, 7, 13, 15, 23, 50, 53, 55 days	6·4 gm.	11, 21, 31 days	Lived 60 days. From 31st to 54th day Tryps. present; absent at death. Cause of death—Haemorrhagic Nephritis.
245	,,	8 k. 700 grs.	Aug. 7	4, 5, 6, 7 days	1·1 gm.	—	Lived 14 days. Tryps. absent at death. Cause of death—Haemorrhagic Nephritis.

II. *p.* AMINO-PHENYL-STIBINIC ACID

After the somewhat discouraging results obtained with the use of *m.* Amino-phenyl-stibinic acid, experiments were undertaken with a view to ascertaining whether the *p.* compound was superior in its action to the *m.* compound.*

A. Rats.

Rats infected with *T. brucei*, *T. evansi*, and *T. gambiense* were used in the following experiments:—

The strain of *T. gambiense*, with which the experiments were carried out, was an especially virulent strain. It was recovered from a monkey at the time of its last relapse, a few days before death. Rats succumbed to the infection, on an average, 3-4 days after inoculation.

As a routine method of treatment, after some preliminary experiments, the following procedure was decided upon:—1st day, one injection of 0·5 c.c. of 5% solution, followed on the 3rd day by 0·25 c.c. of 5% solution. The injections were repeated after a varying interval, as seen in Table III, pp. 374-375.

This mode of treatment was found to be superior to injections of 0·5 c.c. of 5% solution on two subsequent days. Only a small percentage of the rats succumbed to the poisoning effects of Antimony, which took the form of a severe diarrhoea. At the post-mortem the mucous membrane of the intestine was markedly oedematous and inflamed; the kidneys showed all the signs of an acute inflammation.

The effect of the injection of the compound on the trypanosomes was very marked. The parasites disappeared usually after 12-16 hours. If treatment was discontinued the parasites reappeared in a comparatively short time, and a further injection again caused their prompt disappearance. Occasionally, after repeated injections the interval between relapses became shorter and shorter, until, finally, an injection of the drug had no influence at all on the parasites, and the animal died from trypanosomiasis. Table IV gives the details of these experiments. We were able to confirm Mesnil's and Brimont's[12]

* It is a very interesting observation of Ehrlich's that the *m.* Amino-phenyl-arsenic acid is markedly inferior in its therapeutic value to the *p.* Amino-phenyl-arsenic acid (Atoxyl). (Private communication.)

observation that this strain is not resistant to the drug in the same sense as Atoxyl resistant strains. If subinoculated into new rats, an injection of the Antimony compound caused a prompt disappearance of these parasites from the blood.

B. Dogs.

Experiments with dogs infected with *T. evansi* and *T. brucei* showed that these animals are very susceptible indeed to the toxic effect of the *p.* Antimony compound. If small doses were administered, the parasites did not disappear from the blood. If the doses were increased, the animal died in a very short time with severe kidney lesions. At the post-mortem, the kidneys were swollen and congested; subcapsular and cortical haemorrhages were noticed. The urine was of a slight reddish colour, containing red blood corpuscles, casts, and large quantities of albumen. Table V, p. 377, gives the details of these experiments.

Preliminary experiments on guinea-pigs proved that these animals reacted to the *p.* compound in the same way as to the *m.* compound.

C. Monkeys.

Monkeys infected with *T. gambiense* were used for the following experiments. Treatment was usually begun when the infection was well established, and the animals presented undoubted signs of illness. Two monkeys were treated at a late stage of the infection, two at an earlier stage with the *p.* compound. Two others were treated with a combination of *p.* amino-phenyl-stibinic acid and Atoxyl.

Experiment No. XV.—*Cercopithecus callitrichus*, weight 2 k. 920 gm. Treatment was begun seventy-two days after infection. Numerous parasites were then found in the blood. The animal was injected with 0·2 gm. of the *p.* compound. The parasites had disappeared by the next morning. Symptoms of Antimony poisoning had, however, set in: the monkey was vomiting white, slimy masses, and was suffering from severe diarrhoea; the eyes being congested. The next day tremors were noticed all over the body; the eyes were glassy and staring; the mucous membrane of the mouth was cyanotic; the temperature was subnormal, 95°; the blood was dark and contained numerous leucocytes. These symptoms increased; and the animal died in the evening of the following day.

At the post-mortem, numerous subpleural haemorrhages were found; the lungs were normal; the heart pale and soft; the liver was very pale, and showed typical signs of a parenchymatous degeneration; the spleen was enlarged; the kidneys were pale and slightly congested; the medullary and cortical substances not well defined; the mucous membrane of the stomach was congested, and that of the intestines oedematous.

TABLE III.

Exp. No.	Disease	Date of inoculation	Treatment at the end of—	c.c. of 5 % solution	Relapse after	Remarks
266 A.	*T. gambiense*	Dec. 24	3, 10, 23, 42 days	1·75 c.c.	10 days	Animal living at the end of 93 days.
B.	,,	,,	3 days	0·5 c.c.	—	Lived 4 days. Tryps. absent at death.
C.	,,	,,	3, 10 days	0·75 c.c.	—	Animal still living at the end of 93 days.
267 A.	,,	Dec. 28	3, 13 days	0·75 c.c.	—	Lived 21 days. Spleen small.
B.	,,	,,	,,	0·75 c.c.	—	,, 33 ,, Died of intercurrent diarrhoea.
C.	,,	,,	,,	0·75 c.c.	—	,, 33 ,, ,, ,, ,,
D.	,,	,,	,,	0·75 c.c.	—	,, 21 ,, Spleen small.
278	,,	Jan. 20, 1909	3 days	0·5 c.c.	—	Lived 4 days. Kidneys inflamed, diarrhoea.
360 A.	,,	Jan. 3	4 days	0·5 c.c.	—	Treatment started 6 hrs. before death. Tryps. still present at death, decreased in number.
B.	,,	,,	,,	0·5 c.c.	—	,, ,, ,,
363 A.	,,	Jan. 7	2, 4 days	0·75 c.c.	—	Lived 7 days. Died of abscesses of the lung.
B.	,,	,,	,,	0·75 c.c.	—	,, 6 ,, ,, ,, ,,
372	,,	Jan. 14	5, 14, 22, 38 days	2 c.c.	14, 21, 38	,, 48 days. Tryps. absent at death.
382 A.	,,	Jan. 27	2 days	0·5 c.c.	—	,, 3 ,, ,, ,,
B.	,,	,,	,,	0·5 c.c.	—	,, 6 ,, ,, ,,
271 A.	*T. brucei*	Jan. 4	5, 7, 14 days	1·25 c.c.	—	,, 23 days. Tryps absent at death. Spleen enlarged.
B.	,,	,,	3, 5, 16 days	1·25 c.c.	16 days	,, 31 ,, ,, ,,
C.	,,	,,	3, 5, 16, 28 days	1·75 c.c.	28 days	,, 29 ,, ,, ,,
D.	,,	,,	3, 5 days	1·75 c.c.	—	,, 6 ,, ,, ,,
E.	,,	,,	3, 5, 16 days	1·25 c.c.	—	,, 21 ,, ,, ,, Spleen small.
F.	,,	,,	3, 5, 16 days	1·25 c.c.	—	Animal still living at the end of 86 days.

272 A.	*T. brucei*	Jan. 4	3, 5 days	0·75 c.c.	—	Lived 18 days. Tryps. absent at death.
B.	,,	,,	5 days	0·5 c.c.	—	,, 5 ,,
C.	,,	,,	5 days	0·5 c.c.	—	,, 6 ,, Tryps. absent at death. Spleen enlarged; marked Haemoglobinuria.
D.	,,	,,	3, 5, 7 days	1·0 c.c.	—	,, 12 ,, Tryps. absent at death.
E.	,,	,,	5, 7 days	0·75 c.c.	—	Animal still living at the end of 86 days.
F.	,,	,,	Control	—	—	Died at the end of 5 days.
322 A.	,,	Nov. 28	5, 7 days	1·0 c.c.	—	Lived 7 days. Kidneys inflamed; mucous membrane of intestine oedematous.
B.	,,	,,	,,	1·0 c.c.	—	,, 7 ,, ,, ,, ,, ,,
329 A.	,,	Dec. 3	2 days	0·5 c.c.	—	,, 5 ,, Tryps. absent at death.
B.	,,	,,	,,	0·5 c.c.	—	,, 5 ,, ,, ,,
332 A.	,,	Dec. 5	3 days	0·5 c.c.	—	Animal still living at the end of 115 days.
B.	,,	,,	,,	0·5 c.c.	—	Lived 4 days.
365 A.	,,	Jan. 9	4, 38 days	1·0 c.c.	36 days	Lived 40 days.
B.	,,	,,	3 days	0·3 c.c.	—	,, 3 ,,
325	*T. evansi*	Nov. 30	5, 7 days	0·75 c.c.	15 days	,, 17 days. Died from Trypanosomiasis.
362 A.	,,	Jan. 7	2, 4, 20, 30, 35, 42, 45, 49 days	3·5 c.c.	20, 28, 33, 41-56	,, 56 ,, ,, ,,
367 A.	,,	Jan. 9	3, 4 days	1 c.c.	—	,, 6 ,, Tryps. absent at death.
B.	,,	,,	,,	0·8 c.c.	—	,, 5 ,, ,, ,,

Table IV.

Rat Experiment 362.—

Inoculated with *T. brucei*, January 7.

January	8.	10-15 to field	0·5 c.c. of 5 % *p.* compound
	9.	Numerous.	
	10.	Neg.	
	11.	Neg.	0·25 c.c. „ „ „
	26.	Neg.	
	27.	1 to 5 fields	0·5 c.c. „ „ „
	28.	1-5 to field.	
	29.	Neg.	
February	3.	Neg.	
	4.	1 to ½ film.	
	5.	1 to 20 fields.	
	6.	5 to field.	0·5 c.c. „ „ „
	7.	Neg.	
	8.	Neg.	
	9.	1 to ¼ film.	
	10.	1 to 2 fields.	
	11.	10 to field	0·5 c.c. „ „ „
	12.	1 to 20 fields.	
	13.	Neg.	
	17.	Neg.	
	18.	1 to ½ film.	
	19.	1 to 10 fields	0·5 c.c. „ „ „
	20.	5 to field.	
	22.	15 to field	0·5 c.c. „ „ „
	23.	15 to field	0·5 c.c. „ „ „
	24.	1 to ½ film.	
	25.	1 to field.	
	26.	5 to field	0·5 c.c. „ „ „
	27.		
	28.		
March	1.	Increasing	
	2.	in number.	
	3.		
	4.	50 to field.	
		Dead.	

TABLE V.

Exp. No.	Disease	Weight	Date of inoculation	Treatment at the end of—	c.c. of 10% solution	Relapse after days	Remarks
268	*T. evansi*	10 k. 850 grs.	Jan. 2	16, 19 days	6 c.c., 3.cc.	—	Lived 23 days. Tryps. absent from peripheral blood at death. Cause of death—Haemorrhagic Nephritis.
269	*T. evansi*	6 k. 100 grs.	Jan. 2	13 days	3 c.c.	—	Lived 15 days. Tryps. present in the blood at death. Cause of death—Haemorrhagic Nephritis.
270	*T. evansi*	6 k. 150 grs.	Jan. 2	13, 16 days	3 c.c., 3 c.c.	—	Lived 20 days. Tryps. absent from peripheral blood at death. Cause of death—Haemorrhagic Nephritis.
280	*T. brucei*	8 k. 500 grs.	Jan. 25	5, 13 days	3 c.c., 6 c.c.	—	Lived 15 days. Tryps. absent from the peripheral blood at death. Cause of death—Haemorrhagic Nephritis.

EXPERIMENT XXVI.—*Macacus rhesus*, weight 2 k. 245 gm. Treatment was begun on the twentieth day after inoculation. The animal was then in a very advanced stage of the disease. The face was puffy, the genitals swollen and oedematous. The blood count gave 1,570,000 red cells, 3,700 white cells and haemoglobin 55 per cent. 0·1 gm. of *p*. compound was then injected. The parasites disappeared about eleven hours after the injection, but the animal was found dead next morning in its cage. The post-mortem revealed the typical lesions of an advanced trypanosomiasis in monkeys.

Two monkeys were inoculated at an earlier stage of the disease.

EXPERIMENT XX.—*Macacus rhesus*, weight 2 k. 540 gm., was injected on the fifteenth day after inoculation with 0·1 gm. of *p*. compound; a relapse set in nine days after the injection, when the same dose was repeated. Thirty-two days afterwards, a third injection of 0·1 gm. of the drug was administered, and then treatment was discontinued. The animal is still alive on the ninety-eighth day after inoculation, and has increased in weight to 2 k. 720 gm. The period of observation is, however, far too short to consider this animal cured.

EXPERIMENT XXIV.—*Macacus rhesus*, weight 1 k. 650 gm. Injected with *T. gambiense*. Very soon the animal showed oedema of the eyelids and oedematous swelling of the genitals. Treatment was begun thirteen days afterwards with an injection of 0·1 gm. of the *p*. compound. The parasites had disappeared from the peripheral circulation by the next day. The same dose was repeated on the twenty-first and thirty-seventh day after inoculation; the treatment was then discontinued. The animal is still alive, sixty-four days after inoculation, and has increased in weight to 1 k. 720 gm.

In order to ascertain the value of a combined Antimony-Atoxyl treatment in monkeys infected with *T. gambiense*, two monkeys (*Macacus rhesus*) were used in the following experiments:—

EXPERIMENT XXII.—*Macacus rhesus*, weight 2 k. 190 gm. The animal was injected twenty-three days after inoculation with 0·1 gm. of *p*. compound. The parasites disappeared promptly from the peripheral circulation. This was followed on the thirty-first day by an injection of 0·1 gm. of Atoxyl. On the forty-eighth day the injection of 0·1 gm. of the *p*. compound was repeated. The animal is still alive, sixty-two days after inoculation, and has regained its original weight.

EXPERIMENT XXV.—*Macacus rhesus*, weight 1 k. 985 gm. It was treated in the same way as in Experiment XXII. This animal is still alive, but in both cases the observation time is far too short to pronounce the animals cured.

CONCLUSIONS

1. The foregoing experiments prove that *p*. and *m*. amino-phenyl-stibinic acids are fairly powerful trypanocides, although their action is not so rapid as that of Sodium-antimonyl-tartrate.

2. That the *p*. amino-phenyl-stibinic acid is decidedly superior in its action to the *m*. amino-phenyl-stibinic acid.

3. Considering the satisfactory results obtained in experimental animals, a trial of the *p*. amino-phenyl-stibinic acid in patients suffering from Sleeping Sickness is justifiable.

4. In our opinion *p*. amino-phenyl-stibinic acid may be administered in the same doses as Atoxyl. As kidney lesions are among the most pronounced results of Antimony poisoning a careful systematic examination of the urine is advisable.

APPENDIX

For the preparation of *p.* and *m.* amino-phenyl-stibinic acid we used the method previously adopted by Michaelis[13] for the production of Di-methyl-amino-phenyl-arsenic acid, employing, however, antimony trichloride instead of arsenic trichloride.

Antimony trichloride was treated with aniline, and the compound $H_2N.\ C_6\ H_5.\ SbCl_2$ obtained, which changed in the presence of alkali into the corresponding hydroxide. This formed, on treatment with hydrogen peroxide in an alkaline solution, aryl-stibinic acid. The process is expressed chemically as follows:—

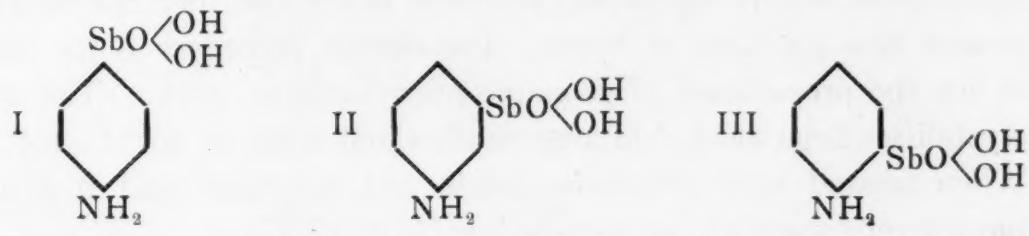
$$\text{(I)}\quad H_2N \cdot C_6H_5 + SbCl_3 = H_2N \cdot C_6H_4 \,.\, SbCl_2 + HCl$$

$$\text{(II)}\quad H_2N \cdot C_6H_4 \cdot SbCl_2 + 2KOH = H_2N \cdot C_6H_4 \cdot Sb\left\langle\begin{matrix}OH\\OH\end{matrix}\right.$$

$$= H_2N \cdot C_6H_4 \cdot SbO \cdot + H_2O$$

$$\text{(III)}\quad H_2N \cdot C_6H_4 \cdot SbO + H_2O_2 = H_2N \cdot C_6H_4 \cdot SbO\left\langle\begin{matrix}OH\\OH\end{matrix}\right. \text{(Arylstibinic acid)}$$

It is a remarkable fact that on adding aniline to *melting* antimony trichloride, *p.* amino-phenyl-stibinic acid (I) is formed, while, on the other hand, if antimony trichloride is added to *boiling* aniline, *m.* amino-phenyl-stibinic acid (II) is obtained.



For the preparation of *o.* amino-phenyl-stibinic acid (III) we used *Grignar's* reaction, treating *o.* chloraniline with antimonic acid.

Preparation of p. amino-phenyl-stibinic acid.

Thirty grammes of antimony trichloride are heated to 205° in a carefully dried flask for about 6-10 minutes, and 30 grammes of aniline added in three portions. The mixture is kept boiling for 15 minutes and then poured into 500 c.c. of water. The precipitate which is formed is collected on a filter paper and then added to a hot solution of sodium carbonate, which dissolves free antimonic acid. It is then boiled with 80 c.c. of strong potassium hydrate (25%) for three

hours, diluted, and 75 c.c. of commercial hydrogen peroxide added. At the end of two days the precipitate is boiled for two hours and filtered. On acidifying with diluted sulphuric acid, the *p.* amino-phenyl-stibinic acid is then obtained, which crystallizes in small needles from alcohol and water (1 : 3) and readily forms a sodium salt. The latter does not melt under 360° C., but turns brown at 323° C.-326° C. When treated with potassium iodide and sulphuric acid it is easily converted into *p.* iodo-aniline (M.P. 61° C.).

Below is an analysis of the sodium salt.

($C_6H_4O_3NSbNa$)

	Calculated	Found	(2)
C	25·26 per cent.	24·37 per cent.	25·17 per cent.
H	2·80 ,,	3·04 ,,	2·92 ,,
N	4·91 ,,	5·18 ,,	5·04 ,,
Sb	42·11 ,,	42·63 ,,	42·43 ,,
Na	8·06 ,,	8·19 ,,	8·27 ,,

Preparation of m. amino-phenyl-stibinic acid.

Twelve grammes of aniline are kept boiling, using an air condenser, and fifteen grammes of antimony trichloride are added in small portions. After all the antimony trichloride is dissolved, the temperature is kept up to 165° for three hours, and then the mixture poured into 500 c.cm. of water. The further procedure is the same as for the preparation of *p.* amino-phenyl-stibinic acid. The acid crystallizes from alcohol in long needles and melts at 207° C.-208° C When treated with potassium iodide and sulphuric acid it forms, though very sparingly, *m.* iodo-aniline (M.P. 26° C.).

It is easily acetylated on boiling with acetic anhydride for two hours, and yields small needles which crystallize from diluted alcohol (1 : 15). M.P. 186° C.-188° C.

Below is an analysis of the sodium salt of *m.* amino-phenyl-stibinic acid:—

($C_6H_4O_3NSbNa$)

	Calculated	Found (1)	(2)
C	25·26 per cent.	25·03 per cent.	—
H	2·80 ,,	3·12 ,,	—
N	4·91 ,,	4·72 ,,	—
Sb	42·11 ,,	42·76 ,,	42·26 per cent.
Na	8·06 ,,	8·14 ,,	8·19 ,,

Preparation of o. amino-phenyl-stibinic acid.

Two grammes of *o.* chlor-aniline are dissolved in 30 c.c. of *dry* ether, and one gramme of antimonic acid added. After carefully drying for two hours at 200° C., 0·4 gramme of magnesium is added. The temperature rises to 45° C.. and is kept at this temperature for 1-1½ hours. After evaporation of the ether, the residue is shaken up with 25 c.c. of sodium carbonate solution (5%), and the mixture is then warmed on a steam bath for one hour and filtered. The filtrate is acidified with diluted sulphuric acid, and the resulting precipitate dried and extracted with absolute alcohol. On evaporation of the alcohol, *o.* amino-phenyl-stibinic acid is left, which crystallizes from a mixture of alcohol and pyridine (1 : 3) in leaflets. The *o.* amino-phenyl-stibinic acid melts at 192° C. to 194° C., and forms an acetyl derivative on boiling with acetic anhydride (M.P. 167° C.-169°C.). On treatment with potassium iodide and sulphuric acid it yields *o.* iodo-aniline (M.P. 54° C.).

($C_6H_4O_3NSbNa$)

	Calculated	Found
C	25·26 per cent.	25·12 per cent.
H	2·80 ,,	2·93 ,,
N	4·91 ,,	5·16 ,,
Sb	42·11 ,,	42·76 ,,
Na	8·06 ,,	8·23 ,,

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SHORT NOTE ON THE MECHANISM OF HAEMOLYSIS IN *PIROPLASMOSIS CANIS*

BY

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(Received for publication 6 April, 1909)

For the following researches puppies and dogs of various ages were infected with our laboratory strain of *Piroplasma canis.* The infection was invariably fatal for *young* puppies up to the age of $2\frac{1}{2}$ months. After a varying incubation period, the animals shewed parasites in the blood in small numbers at first, but very soon a rapid multiplication occurred and lasted for 24 to 36 hours, accompanied by haemoglobinuria. The urine was of a light reddish to dark mahogany colour, and the more rapid the multiplication of the parasites, the darker was the colour of the urine. Pronounced jaundice of the tissues was only noticed in two out of seventy animals used. In these young animals the blood serum, taken at death, was of a dark reddish to mahogany colour, according to the colour of urine.

In dogs and older puppies, the disease was hardly ever fatal. The parasites did not multiply so rapidly, and nearly always were present in smaller numbers; we have never observed a pronounced haemoglobinuria in these animals, but for three weeks after inoculation the serum was slightly reddish in colour.

It was especially noticed that in young puppies a rapid diminution of the blood corpuscles took place during the last few hours before death.

It seemed, therefore, of interest to determine whether the destruction of the blood corpuscles was due to Isolysins or Autolysins. With this object, the serum of infected animals in different stages of the disease was added in varying dilutions to red blood corpuscles of

the infected animals from which the serum was obtained, and also to blood corpuscles of normal dogs or puppies.

The serum was used both fresh and after being heated for thirty minutes at a temperature of 56° C.; and in this latter case with and without the addition of complement (fresh centrifugalised dog serum).

The blood corpuscles were usually washed three times in 0·9% saline solution, and used in a 10% suspension.

Serum of a dark reddish colour taken from a puppy three hours before death: haemoglobinuria marked.

	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
A. 10 per cent. suspension of infected dog's red blood corpuscles	1	1	1	1	1	1
Fresh normal dog's serum (diluted 1 in 10) ...	1	1	1	1	1	1
Infected dog's inactivated serum	1	0·5	0·1	0·05	0·01	0·005
Two hours at 37° C. ...	Slight Haemolysis	0	0	0	0	0
B. 10 per cent. suspension of infected dog's blood corpuscles	1	1	1	1	1	1
Infected dog's serum ...	1	0·5	0·1	0·05	0·01	0·005
Two hours at 37° C. ...	Slight Haemolysis	0	0	0	0	0
C. 10 per cent. suspension of infected dog's blood corpuscles	1	1	1	1	1	1
Normal serum	1	0·5	0·1	0·05	0·01	0·005
Two hours at 37° C. ...	0	0	0	0	0	0
D. 10 per cent. suspension of normal dog's blood corpuscles	1	1	1	1	1	1
Fresh normal dog's serum (diluted 1 in 10) ...	1	1	1	1	1	1
Inactivated infected dog's serum	1	0·5	0·1	0·05	0·01	0·005
Two hours at 37° C. ...	0	0	0	0	0	0

	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
E. 10 per cent. suspension of normal dog's blood corpuscles	1	1	1	1	1	1
Infected dog's serum ...	1	0·5	0·1	0·05	0·01	0·005
Two hours at 37° C. ...	0	0	0	0	0	0

Similar experiments were carried out with the serum of a larger dog, withdrawn during the early acute stage of the infection, when parasites were present in small numbers. The serum was of a light reddish colour. The results were entirely negative: in none of the test tubes was there any sign of haemolysis.

The series of experiments was repeated in the same way as in the first experiment, with the serum of a puppy; the serum of which was of a dark brownish mahogany colour; and also in these experiments, 1 c.c. of the infected serum added to 1 c.c. of 10% infected blood corpuscles, with both activated and inactivated serum, caused an extremely slight haemolysis, whereas in no other tube was there any sign of haemolysis noticed.

The same experiment was repeated with the serum of a dog three weeks after the first appearance of the parasites, and also with the serum of a puppy at the commencement of the rise of temperature. In no case was there any haemolysis of either normal red blood corpuscles, or of the red corpuscles of the infected animal from which the serum was obtained.

CONCLUSION

These experiments tend to prove that the haemolysis and the haemoglobinuria in infections with *Piroplasma canis* is due neither to an Isolysin nor to an Autolysin, but apparently only to a disintegration of red blood corpuscles after the escape of the parasites from them.

GLAND PUNCTURE IN THE DIAGNOSIS OF ANIMAL TRYPANOSOMIASIS

BY

R. EUSTACE MONTGOMERY, M.R.C.V.S.,

AND

ALLAN KINGHORN, M.B.,

(Received for publication 15 *April*, 1909*)*

Acting on a suggestion made by Mott, Greig and Gray examined the juice obtained by puncture of the enlarged lymphatic glands of men suffering from trypanosomiasis in Uganda. Dutton and Todd in the Congo Free State also noted their connection with the disease, and instituted a system of census dependent upon their size. They found that 91% of natives having post-cervical glands measuring approximately 1·5 by 0·75 cm. showed trypanosomes on puncture.

Enlargement of the superficial lymphatic glands in animals, though well recognised post-mortem, has not received much attention as a symptom of trypanosomiasis. Dutton, Todd and Kinghorn* quote Bertolloti as having noted it to be a constant feature in the infected stock at Eala; and enlargement of the presternals is well known to camel owners in the Punjab, and by some at least is associated with Surra. In Rhodesia we found it to be common in all classes of animals sick and healthy, even young calves and several varieties of antelope having almost without exception easily palpable glands. As a symptom, then, it is here of little value.

During the time one of us (R. E. M.) was in India, a few observations were made as to the value of gland puncture in camels believed to be suffering from Surra, but not showing trypanosomes in the peripheral circulation. In one case a camel which did not show peripheral organisms for sixty-three consecutive days revealed them on three out of four punctures; and on another occasion when examining a herd, two additional cases were discovered by this method.

* Annals of Tropical Medicine and Parasitology, 1907, Vol. 1, p. 235.

Our observations on cattle suffering from *T. dimorphon* at Broken Hill* did not accord with those of Dutton and Todd on Congolese cattle. The infection in our animals, however, was acute, and trypanosomes were rarely absent from the circulation: in such cases it is unnecessary to resort to gland puncture. Our more recent observations on animals suffering from a somewhat chronic form of disease leads us to regard it as a most valuable diagnostic method. It is not only of value in chronic cases, but should animal inoculation have been carried out, a positive examination may be obtained some days prior to the appearance of trypanosomes in the blood. We may quote the following cases:—

A goat inoculated with a cattle strain of *T. dimorphon* showed trypanosomes on gland puncture from the ninth day: they only appeared in the blood (1 in ¼ cover-glass) on the fourteenth.

A dog inoculated with the same strain never showed peripheral trypanosomes up to death on the thirty-fifth day. Gland puncture on the tenth and nineteenth days was positive.

An ox inoculated with a trypanosome allied to *T. brucei* showed organisms in the prescapular glands two days prior to their appearance in the blood.

An ox inoculated with a trypanosome of doubtful nature (Ninamwenda strain) showed organisms in the gland three days before they were seen in the blood.

A dog inoculated with a dog strain of tadpole trypanosomes showed organisms on gland puncture eleven days previous to their being seen in the blood.

Infections of the goat due to *T. brucei* and *T. pecaudi* are known to be easily overlooked, unless means of sub-inoculation are at hand. The following cases are instances of the advantages of puncture:—

A goat inoculated with a form allied to *T. brucei*, which was under observation for six weeks, only showed trypanosomes on two occasions. They were present on each of five gland punctures when not seen in the blood.

A goat inoculated with the same strain did not show blood trypanosomes during the fortnight available for observation. Gland puncture showed them present from the seventh day.

It is, of course, hardly to be expected that success is invariable. In animals known to be infected we have often been unable to detect their presence on puncture; but the advantages of even one positive case fully compensate for the small amount of labour involved in adopting this method as a routine for all suspected animals not showing peripheral trypanosomes.

The advantages in practice may be judged by the fact that in three localities fifteen cases were diagnosed on blood examination, and four additional ones on puncture; whilst at another place,

* Annals of Tropical Medicine and Parasitology, 1908, Vol. 2, p. 106.

considered to be free of disease, it was by gland puncture alone that its existence was demonstrated.

We are indebted to Mr. Lane, the Veterinary Officer of North Eastern Rhodesia, for the following summary of results since adopting this method of diagnosis. The trypanosome with which he had chiefly to do is, in animal reaction and morphology, related to *T. nanum*. On one Station four cases were diagnosed by gland puncture: in no instance were trypanosomes seen in the blood. On a second Station, in five cases the gland juice was positive, while only two showed organisms in the blood.

OBSERVATIONS ON THE HOOKLETS OF *CYSTICERCUS CELLULOSAE* IN MAN

BY

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WALTER MYERS LECTURER IN TROPICAL MEDICINE

(Received for publication 23 April, 1909)

In May, 1908, Drs. Campbell and Thomson, of Jammalamadagu, Madras, kindly presented to the museum a specimen of *Cysticercus cellulosae* in the pectoral muscle of man. The size of the connective

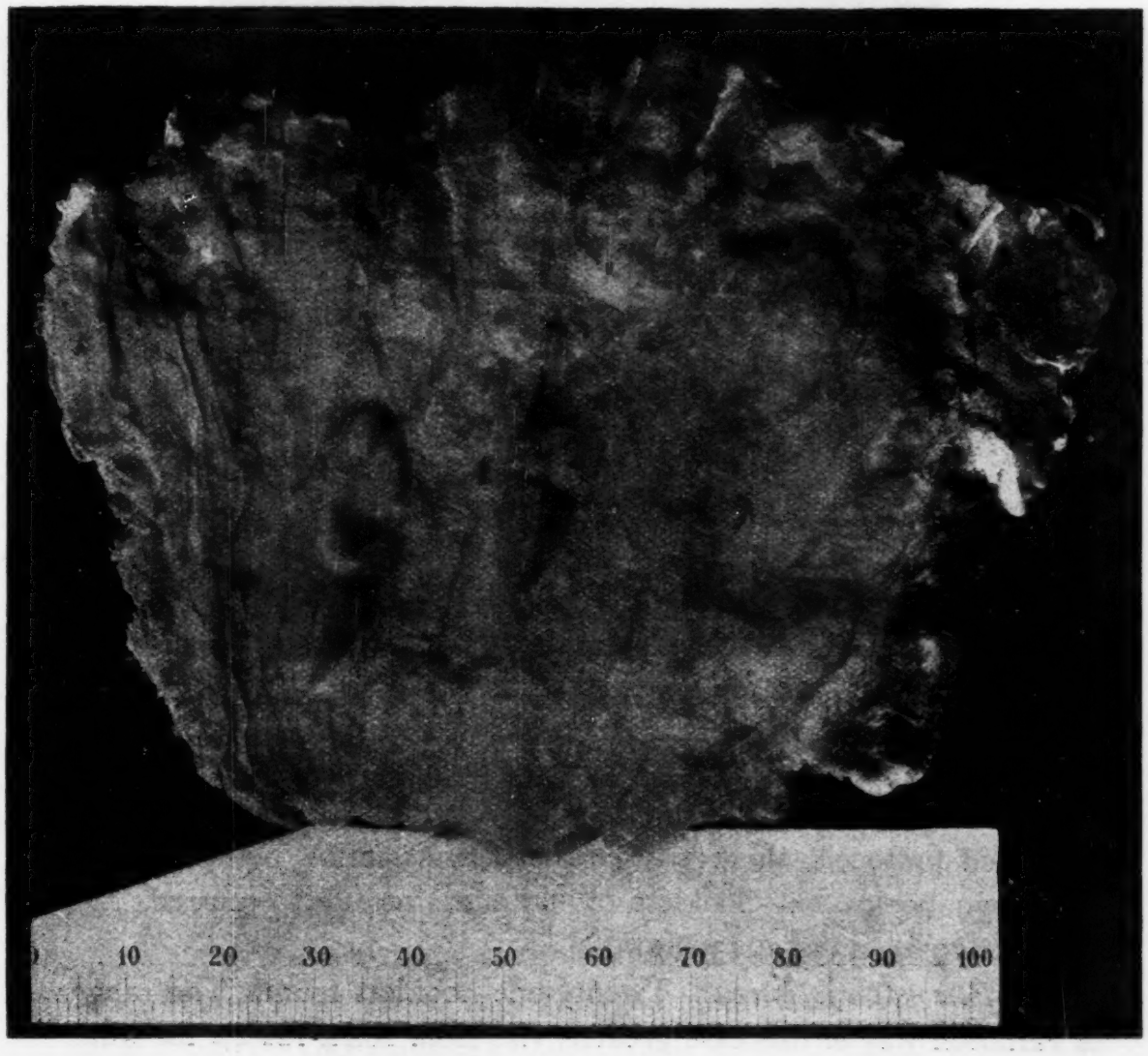


FIG. 1.—*Cysticercus cellulosae.* Pectoral muscle of man. Natural size.

tissue capsules of the cysts varied from 15-21 mm. long by 8-10 mm. broad (fig. 1). Recently I proceeded to examine a scolex extracted from its bladder with a view to making certain of the diagnosis. I was surprised accordingly, on examining a specimen, to find only sixteen hooklets instead of twenty-two to thirty-two, which is the number given by various authorities as comprising the limits of variation. It was possible that one circle of hooklets was absent, but on measuring, this explanation, taken also in connection with what will appear later, is hardly possible.

FIG. 2.—Circle of hooklets of the same. × 80 approximately.

1. *Pectoral cysticercus* (fig. 2). Sixteen hooklets. The size of the hooks varied from 108·0(?)μ-144·0μ. As will be seen from the appended protocols, there was no sharp demarcation between small and large hooks, but hooklets of various sizes also occurred, *e.g.*, 126·6μ, 129·6μ, 133·2μ, 136·8μ.

2. *Pectoral cysticercus.* Twenty-one hooklets found. The range of variation was in this case greater, viz., from 104·4-122·4μ for what might be called small hooks, and from 154·8-165·6μ for the large.

3. *Pectoral cysticercus.* Hooklets twenty. Only a few hooks were measured, three small, varying from 108·0-111·6μ, and five large, varying from 144·0-151·2μ.

4. *Pectoral cysticercus.* Twenty hooklets. In this case, as in case No. 1, it is hardly possible to separate the hooks into a large and small series, as hooks of an intermediate size occur. Thus hooks of the following sizes were measured 122·5, 129·5, 133·0, 140, 143·5, 147·0, 150·5, 153·5μ.

I next examined a specimen of *C. cellulosae* from the brain and a specimen from the tongue; both from natives of Madras and presented

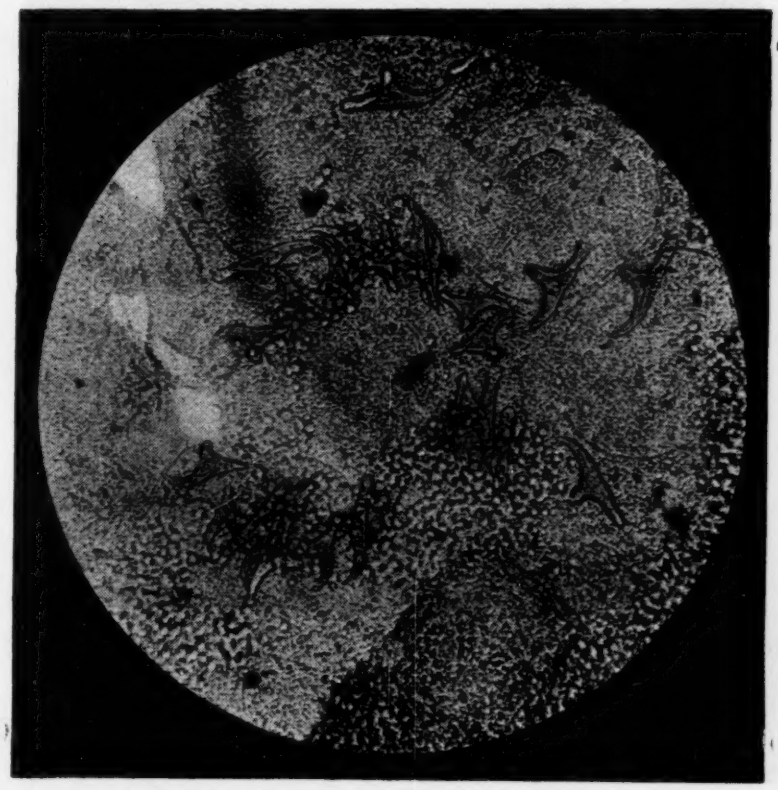


FIG. 3.—*Cysticercus cellulosae* from brain. × 80 approximately.

to the museum by Major Williams, I.M.S., and compared them with the hooks of *T. solium* in man and *C. cellulosae* from the pig.

5. *Brain cysticercus* (fig. 3). Hooklets twenty-eight. The 'small' range from 104·6-118·8μ; the 'large' from 151·2-162·0μ, so that there is a fairly well marked line of separation.

6. *Tongue cysticercus* (fig. 4). One month's duration. Hooklets twenty-two (? two missing). The small range from 108·0-122·4μ. The large from 140·4-151·2μ. The range of variation is not so great,

nor is the line of separation between the small and large so marked as in the brain cysticercus.

7. *Pig muscle cysticercus.* Twenty-five hooklets found. Ten hooklets were measured. The size of the small was constant, viz., 126μ. That of the large was also constant, viz., 175μ, so that separation between large and small was quite distinct.

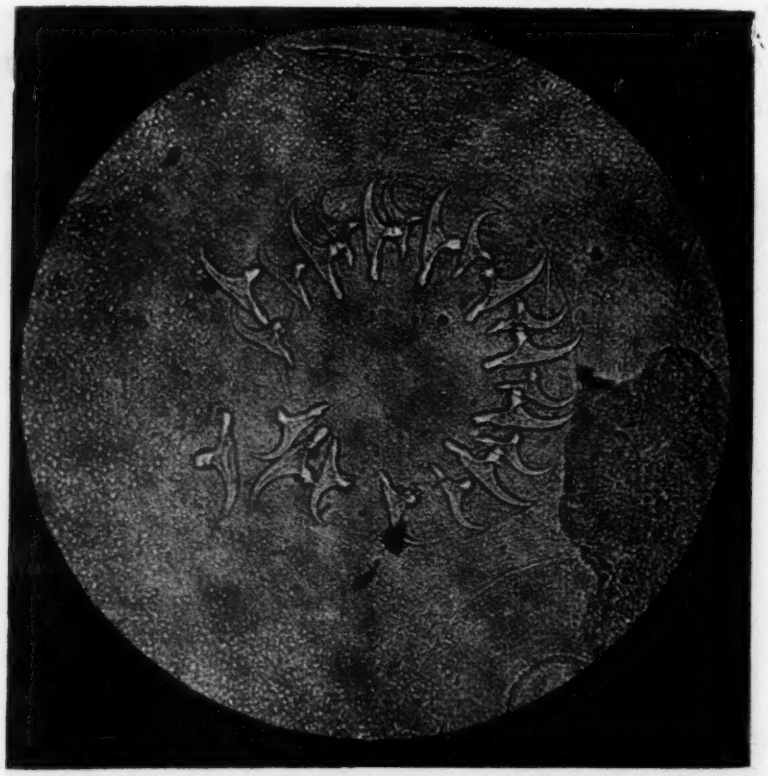
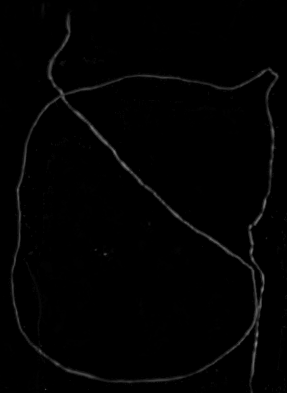


FIG. 4.—*Cysticercus cellulosae* from tongue. × 80 approximately.

8. *T. solium.* Twenty-five hooklets were found. Eighteen of these were measured. The small range from 115·2-140·4μ and the large from 183·6-187·2μ, so that the line of separation is again distinct, though it is noticeable that the size of the hooklets is distinctly larger than in the case of the cysticercus in the pig's muscle.

It would appear, therefore, from these observations that in *C. cellulosae* in man there is an irregularity of development affecting both the number of the hooklets and, more especially, their size.

1	2	3	4	5	6	7	8
T. solium Hooklets 25	Brain cyst. Hooklets 28	Tongue cyst Hooklets 22 (? 24)	Pectoral cyst 1. Hooklets 16	Pectoral cyst 2 Hooklets 21	Pectoral cyst 3. Hooklets over 20	Pectoral cyst 4. Hooklets 20	*C. cellulosae* from pig's muscle. Hooklets 25
1. 115·2	1. 104·6	1. 108·0	1. 108·0	1. 104·4	1. 108·0	1. 122·5	1. 175
2. 118·8	2. 108·0	2. 108·0	2. 126·6	2. 108·0	2. 108·0	2. 129·5	2. 126
3. 118·8	3. 108·0(?)	3. 108·0	3. 126·6	3. 111·6	3. 111·6	3. 129·5	3. 175
4. 122·4	4. 108·0	4. 111·6	4. 126·6	4. 118·8		4. 133·0	4. 175
5. 122·4	5. 108·0	5. 111·6	5. 126·6	5. 118·8	4. 144·0	7. 140·0	5. 175
6. 122·4	6. 108·0	6. 115·2	6. 129·6	6. 118·8	5. 144·0	8. 140·0	6. 126
7. 125·6	7. 108·0	7. 115·2	7. 129·6	7. 122·4	6. 147·6	9. 143·5	7. 126
8. 136·8	8. 108·0	8. 115·2	8. 133·2		7. 151·2	11. 147·0	8. 175
9. 136·8	9. 111·6	9. 115·2	9. 133·2	8. 154·8	8. 151·2	12. 150·5	9. 126
10. 140·4	10. 115·2	10. 118·8	10. 133·2	9. 154·8		12. 150·5	10. 175
	11. 115·2	11. 118·8	11. 133·2	10. 154·8	Small 108·0-111·6	13. 150·5	
11. 183·6	12. 115·2	12. 122·4	12. 136·8	11. 154·8	Large 144·0-151·2	14. 150·5	Small 126μ
12. 183·6	13. 118·8		13. 136·8	12. 154·8		16. 150·5	Large 175μ
13. 183·6	14. 118·8	13. 140·4	14. 144·0	13. 154·8		17. 150·5	
14. 183·6		14. 144·0	15. 144·0	14. 154·8		18. 150·5	
15. 187·2	15. 151·2	15. 144·0		15. 154·8		18. 153·5	
16. 187·2	16. 154·8	16. 147·6		16. 154·8			
17. 187·2	17. 154·8	17. 147·6		17. 158·4		Small 122·5-136·5	
18. 187·2	18. 154·8	18. 147·6		18. 162·0		Large 140-153·5	
	19. 154·8	19. 147·6		19. 162·0			
Small 115·2-140·4	20. 154·8	20. 147·6		20. 165·6			
Large 183·6-187·2	21. 158·4	21. 147·6					
	22. 158·4	22. 151·2		Small 104·4-122·4			
	23. 158·4			Large 154·8-165·6			
	24. 158·4	Small 108·0-122·4					
	25. 158·4	Large 140·4-151·2					
	26. 158·4						
	27. 162·0						
	28. 162·0						
	Small 104·6-118·8						
	Large 151·2-162·0						



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The following changes in the courses of instruction given by the Liverpool School of Tropical Medicine will come into force in and after January, 1909:—

Full Course begins 6 January. Short Course begins 1 June.
Diploma Examination, 5 April. Certificate Examination, 29 June.
Full Course begins 15 September.
Diploma Examination, 13 December.

The full Course of Instruction is open to all qualified medical men, and the examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen guineas.

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1. The Autumn and Lent Courses, which now last only ten weeks, shall be extended to thirteen weeks, followed, as at present, by the examination for the Diploma in Tropical Medicine given by the University.

2. In order to allow of this change being made, the present Summer Term shall be replaced by a short Course of Practical Instruction in Tropical Pathology and Medical Entomology, lasting for four weeks in June, and followed by a class examination with Certificate of Satisfactory Attendance—the acquisition of this Certificate to excuse the first four weeks' attendance for the full Autumn and Lent Courses.

In accordance with this decision during the next year, 1909, the courses of instruction will be given on the following dates:—

Full Course begins 6 January.
Diploma Examination, 5 April.
Short Course begins 1 June.
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Full Course begins 15 September.
Diploma Examination, 13 December.